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Abstract

This dissertation describes a series of toxicological studies carried out in order to better understand the role of monoamine-active drugs in drug-related deaths.

A simple, robust method for determining psychiatric drugs and metabolites was devised using positive mode APESI LC-MS at basic pH. This method was modified to enable determination of multiple commonly abused drugs. It was discovered that both psychiatric drugs and drugs of abuse could be detected using similar chromatographic parameters.

Target drug and metabolite concentrations were determined in postmortem tissue specimens. Drug concentrations were highest in bile and liver and lowest in vitreous humour. Four patterns of distribution were observed, with extent of biliary excretion corresponding to molecular weights. Concentrations in other tissues were compared to those in blood. Several significant correlations were observed, suggesting target drug concentrations in blood could be predicted using these tissues, or could be analysed in lieu of blood in cases of extensive exsanguination or putrefaction.

Antipsychotic concentrations were determined in various brain regions from schizophrenics to determine whether such drugs partition preferentially into particular regions. A further aim was to investigate whether trends exist in how they distribute in brain as a drug class. It was found that if regional drug concentrations were normalised for those in cerebellum, they distributed into three distinct patterns, corresponding to structural features of different phenothiazines. Drug concentrations in certain regions were significantly correlated with selected drug properties, providing the ability to predict their partitioning into such regions. Significant correlations were observed between drug concentrations in selected brain regions and those in blood, suggesting concentrations in these brain regions can be used to predict those in blood, and vice-versa. Evidence was also found to suggest brain distribution of the target antipsychotics is time-dependent, with differing distribution patterns being observable at different points in therapy.

Insufficient data was obtained to investigate postmortem redistribution of individual psychiatric drugs. However, if data for all drugs was combined, concentrations in heart blood were significantly higher than those in femoral blood. A slight difference between heart:femoral blood ratios was observed in cases

where liver drug concentrations were >1.0 mg/kg compared to those with concentrations <1.0 mg/kg. This suggested the observed redistribution was caused by diffusion from solid tissues into surrounding blood.
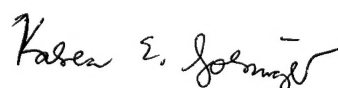
Circumstances were investigated in deaths where target drugs were detected to highlight risk factors associated with their use. There were many cases where contra-indicated drug combinations were detected. In many cases, subjects could have developed serotonin syndrome due to the combination of multiple serotonergic drugs. In several deaths, target drug serotonergic activity may have exacerbated pre-existing medical problems through bleeding disorders (caused by decreased platelet serotonin concentrations) or cardiac vasoconstriction (in subjects with prominent ischaemic damage). No drug-related deaths were attributed solely to a target drug.

In summary, LC-MS was used to investigate a number of toxicological issues associated with monoamine-active drugs. The findings from this research suggest that although such drugs may be safe when taken alone, their toxicity is increased when used in combination with other drugs.

Declaration

The material contained in this dissertation has not been presented for the award of any other degree or diploma in any university or other institution. The research conducted has been carried out solely by the candidate, and this dissertation contains no material previously published or written by another person, except where due reference has been made in the text.

I certify that the writing of this dissertation, with the presented results, interpretations, opinions and suggestions, are entirely my own work.

Kabrena E. Goeringer B.S., M.S.

The Toxicology of Monoamine-active Drugs

A Dissertation submitted for the Degree of

DOCTOR OF PHILOSOPHY

by

Kabrena E. Goeringer B.S. (United States Air Force Academy),

M.S. (University of Washington)

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Southbank, Victoria, AUSTRALIA

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"We are still mad about the mad. We still don't understand them and that lack of understanding makes us mean and arrogant, and makes us mislead ourselves, and so we hurt them."

- David Cohen

"Let conversations cease, let laughter flee;

this is the place where death delights to help the living."

- Inscription on the mortuary wall,

New York City Medical Examiners' Office

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I certify that the writing of this dissertation, with the presented results, interpretations, opinions and suggestions, are entirely my own work.

Kabrena E. Goeringer B.S., M.S.

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Published papers

1. K.E. Goeringer, I.M. McIntyre, O.H. Drummer. "LC-MS analysis of serotonergic drugs". *Journal of Analytical Chemistry* (in press).

2. K.E. Goeringer, I.M. McIntyre, O.H. Drummer. "Postmortem Tissue Concentrations of Venlafaxine". *Forensic Sciences International* 2001; 121: 65-69.

3. B. Dean, G. Pavey, K.E. Goeringer, K. Opeskin, O.H. Drummer, D.L. Copolov, "[^{125}I]Iodosulpride binding to caudate-putamen and pituitary is altered in schizophrenia". *Journal of Neurochemistry* (submitted).

4. K.E. Goeringer, O.H. Drummer. "Case note: postmortem tissue concentrations of risperidone". *TIAFT Bulletin* 2002; 32 (2): pages 9-10.

5. K.E. Goeringer[#], L. Raymon, G.D. Christian, B.K. Logan. "Postmortem forensic toxicology of selective serotonin reuptake inhibitors: a review of pharmacology and report of 168 cases". *Journal of Forensic Sciences* 2000; 45(3): 633-647.

6. K.E. Goeringer[#], L. Raymon, G.D. Christian, B.K. Logan. "Postmortem forensic toxicology of trazodone". *Journal of Forensic Sciences* 2000; 45(4): 850-856.

7. K.E. Goeringer[#], L. Raymon, G.D. Christian, B.K. Logan. "Identification of tramadol and its metabolites in blood from drug-related deaths and drug-impaired drivers". *Journal of Analytical Toxicology* 1997; 21(7): 529-537.

8. K.E. Goeringer[#]. "Serotonergic drugs, their postmortem distribution in man, and their effect on serum serotonin levels". Masters' Thesis, Department of Chemistry, University of Washington, Seattle, Washington, 1996. 147 pages.

Presentations

9. K.E. Goeringer*, G. Pavey, B. Dean, O.H. Drummer. "Schizophrenic brain distribution of selected antipsychotic drugs". Presented at the 2002 Australia/New Zealand Forensic Science Society meeting, Canberra, ACT, Australia, 11-18 May, 2002.

10. K.E. Goeringer*, I.M. McIntyre, O.H. Drummer. "Postmortem tissue concentrations of risperidone". Presented at the 2001 Society of Forensic Toxicologists annual meeting, New Orleans, LA, 29 September-4 October, 2001.

11. K.E. Goeringer*, I.M. McIntyre, O.H. Drummer. "Combined screening and confirmation of drugs of abuse in postmortem specimens using LC-MS following solid-phase extraction". Presented at the 2001 Society of Forensic Toxicologists annual meeting, New Orleans, LA, 29 September-4 October, 2001.

12. K.E. Goeringer*, I.M. McIntyre, O.H. Drummer. "Postmortem tissue concentrations of venlafaxine". Presented at TIAFT 2000 Congress, Helsinki, Finland, 13-17 August, 2000.

13. K.E. Goeringer*, I.M. McIntyre, O.H. Drummer. "LC-MS analysis of psychiatric drugs at high pH". Presented at TIAFT 2000 Congress, Helsinki, Finland, 13-17 August, 2000.

14. K.E. Goeringer[#][*], B.K. Logan. "Review of postmortem toxicology in twenty deaths involving sertraline". Presented at the 1997 Society of Forensic Toxicologists annual meeting, Salt Lake City, Utah, 4-9 October, 1997.

15. K.E. Goeringer[#][*], B.K. Logan. "Postmortem forensic toxicology of trazodone in man". Presented at the 1997 Society of Forensic Toxicologists annual meeting, Salt Lake City, Utah, 4-9 October, 1997.

16. K.E. Goeringer[#][*], B.K. Logan. "Determination of tramadol in blood". Presented at the 1996 Society of Forensic Toxicologists annual meeting, Denver, Colorado, 14-18 October, 1996.

17. K.E. Rodda[†][*], M.W. Ellzy. "Analysis of alkylphosphonic acids using gas chromatography/matrix isolated fourier transform infrared spectrometry (GC/MIFTIR". Presented at the 1994 Scientific Conference on Chemical and Biological Defense Research, ERDEC, Aberdeen Proving Grounds, Maryland, November, 1994.

CONTENTS

APPENDICES

Glossary of terms and abbreviations

Adenylyl cyclase (AC): enzyme acting on ATP to form 3',5'-cyclic AMP plus pyrophosphate.

Affective disorders: disorders of mood.

Affective flattening: restrictions in range and/or intensity of emotional expression, particularly in schizophrenia.

Agonist: a drug capable of combining with receptors to initiate drug actions.

Alogia: restrictions in fluency and productivity of thought and speech, particularly in schizophrenia.

Amphetamines: a class of drugs consisting of a benzene ring and an ethylamine side chain, commonly abused for their stimulant effect.

Akathisia: a side effect of neuroleptic therapy characterised by inability to remain in a sitting posture, with motor restlessness and a feeling of muscular quivering.

Antagonist: a substance that opposes the action of an agonist.

APCI: atmospheric pressure chemical ionization (ionization method used in LC-MS analysis).

APESI: atmospheric pressure electrospray ionization (ionization method used in LC-MS analysis).

Atypical Antipsychotics: neuroleptics associated with fewer extrapyramidal neurological side effects than traditional antipsychotics. These drugs antagonise serotonin activity in addition to that of dopamine.

Autoinhibition: Inhibition of a drug's metabolism by itself. Autoinhibition occurs when a drug is both a substrate for and inhibitor of a particular CYP450 enzyme.

Avolition: restriction in initiation of goal-oriented behaviour, particularly in schizophrenia.

Benzodiazepines: drugs with hypnotic, anxiolytic, and anti-convulsive properties whose structure is composed of a benzene ring fused to a seven-membered diazepine ring.

Bioavailability: relative rate and extent to which an administered drug reaches general circulation; particularly important in oral administration.

BZE: benzoylecgonine, metabolite of cocaine.

Cannabinoids: a class of 61 C_{21} compounds unique to the cannabis plant, some of which are commonly abused for their psychoactive properties.

Catechol-O-methyl-transferase (COMT): enzyme responsible for catalysing metabolism of dopamine, noradrenaline, and adrenaline (in combination with MAO).

Chemical ionisation (CI): ionisation method used in LC-MS in which the analytes of interest are chemically modified to form charged adducts in the ionization chamber which are then detected by the mass spectrometer.

Cholinergic: nerves which use acetylcholine as a transmitter, such as the parasympathetic nerves.

Cirrhosis: interstitial inflammation of an organ, particularly the liver.

Collision-induced Dissociation (CID): a secondary fragmentation method used in conventional LC-MS that expands the sensitivity of the technique.

Cubital fossa: the indentation of the elbow.

Cytochrome P450 (CYP450): the most important family of enzymes that catalyse phase I metabolism of drugs and other substances. A total of 11 CYP450 genes have been found to be involved with metabolism of xenobiotics in humans.

DA: dopamine.

DAD: diode array detection (used with HPLC).

Delusions: distortions in thought content, particularly in schizophrenia.

Depot drugs: decanoate derivatives of neuroleptics with the same pharmacological activity as oral preparations, but which are much longer acting.

Derivatisation: structural modification of analytes of interest to reduce their polarity and thus make them amenable to analysis by GC.

Disorganised speech: distortions in language and/or the thought process, particularly in schizophrenia.

DOM: 4-methyl-2,5-dimethoxyamphetamine (designer amphetamine).

ECD: electron capture detection (used with GC).

Electrospray ionisation (ESI): ionisation method used in LC-MS in which the solvent in charged droplets evaporate, allowing the remaining portion of specimen to pass through the ionization chamber for detection. Requires analytes of interest to be ionised in solution prior to entering the chamber.

EME: ecgonine methyl ester, metabolite of cocaine.

Enzyme multiple immunoassay techniques (EMIT): immunoassays commonly employed by the VIFM.

Epistaxis: profuse bleeding from the nose.

Excitatory postsynaptic potential (EPSP): a localised depolarisation of the postjunctional membrane in ganglionic neurotransmission. GABA modulates the formation of an EPSP.

Extravasation: the act of exuding or passing out of a vessel into the tissues, especially of blood, lymph, or urine.

First-episode cases: subjects presenting with psychotic symptoms with no prior history of schizophrenia.

First-pass metabolism: metabolism of drugs during their first-passes through the liver after oral absorption.

GABA: gamma aminobutyric acid.

Gradient elution: chromatographic method in which mobile phase composition changes over the course of the run.

Haematocrit: red blood cell content in whole blood.

Half-life: approximate time taken to halve drug concentrations in blood during elimination.

Hallucinations: distortions in perception, particularly in schizophrenia.

Haematemesis: vomiting of blood.

5-Hydroxytryptamine (5-HT): chemical name for serotonin.

5-Hydroxyindol-3-acetic acid (5-HIAA): secondary breakdown product of serotonin.

5-HTT: serotonin transporter protein, targeted by SSRIs.

Knockout: genetic modification resulting in a particular gene or receptor subtype not being expressed.

Lipophilicity: Measure of the relative lipid solubility of a drug, expressed as the log octanol/water-partitioning coefficient.

LOD: limit of detection.

LOQ: limit of quantitation.

MS: mass spectrometry (used with either HPLC or GC).

Monoamine oxidase (MAO): enzyme responsible for primary metabolism of serotonin, dopamine, noradrenaline and adrenaline (in combination with COMT).

MDA: methylenedioxyamphetamine (designer amphetamine)

MDE: methylenedioxyethylamphetamine (designer amphetamine)

MDMA: methylenedioxymethylamphetamine (designer amphetamine)

Melanosis coli: abnormal dark brown pigmentation of the large intestinal mucosa due to accumulation of pigment of uncertain composition within macrophages in the intestinal wall.

Modifying agents: solvents added to mobile phases in small concentrations to improve peak shape and/or alter analyte retention.

Monoamines: a class of compounds synthesized *in vivo* which act as neurotransmitters.

NDS: N-desmethylsertraline, active metabolite of sertraline.

Nephrosclerosis: hardening of kidney tissue from overgrowth and contraction of the interstitial connective tissue.

Negative symptoms: schizophrenia symptoms involving diminution or loss of normal functions.

Neuroleptic malignant syndrome (NMS): a syndrome of dopaminergic excess associated with use of neuroleptics and other dopamine-active drugs, resembling a severe form of Parkinsonism.

ODV: O-desmethylvenlafaxine, active metabolite of venlafaxine.

Opioids: a class of commonly abused drugs derived from opium believed to mimic the actions of endogenous compounds with natural analgesic properties.

Phospholipase C (PLC): enzyme that catalyses the hydrolysis of a phospholipid.

pK_a: log acid dissociation constant, a measure of relative compound basicity or acidity.

Positive symptoms: schizophrenia symptoms involving excess/distortion of normal function.

PMMA: para-methoxymethamphetamine (designer amphetamine).

Poor metabolisers: people in whom a particular isoenzyme is not expressed.

Postmortem interval (PMI): time between death and autopsy.

Postmortem redistribution: an occurrence in which drug concentrations in blood in different regions of the body change during the post-mortem interval prior to autopsy.

Protein binding (F_b): extent to which a drug binds to plasma proteins, expressed in percent.

Pulmonary oedema: Swelling of lungs associated with drug toxicity-related CNS depression (particularly opioids).

Recovery: a measure of extraction efficiency, calculated by comparing peak area of an extracted specimen to that of unextracted standards of the same concentration.

Resolution: a measure of separation, calculated by dividing the difference in retention times between two closely eluting compounds by the sum of their corresponding peak widths.

Rhabdomyolysis: disintegration of the striated muscle fibres with excretion of myoglobin in the urine, often as a result of malignant hyperthermia.

SARI: serotonin antagonist reuptake inhibitor (nefazodone is a SARI).

Schizophrenia: mental disorder lasting greater than 6 months which includes at least 1 month of 2 or more positive or negative symptoms.

Selected Ion Monitoring (SIM): a mass spectral detection mode in which only specified ions are monitored during the chromatographic run.

SNARI: serotonin and noradrenaline reuptake inhibitor (venlafaxine is a SNRI).

SSRI: selective serotonin reuptake inhibitor (citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline are SSRIs).

Serotonin syndrome (SS): a syndrome of serotonergic excess associated with the use of serotonin-active drugs stemming from brainstem and spinal cord activation of 5-HT_{1A} receptors.

Solid-phase extraction (SPE): an extraction method that uses cartridges containing organosilane supports to separate analytes of interest from the matrix.

Steatosis: build-up of fatty tissue, especially around the liver.

Stimulants: class of drugs commonly abused for their stimulant effect. Includes amphetamines as well as cocaine.

Sympathomimetic amines: compounds such as the amphetamine-like compounds which act by either replacing monoamine neurotransmitters at, or facilitating release of real neurotransmitters from, nerve endings.

Syndrome of Inappropriate Secretion of Antidiuretic Hormone (SIADH): a common side effect associated with SSRI use manifested by impaired water excretion and associated hyponatraemia and hypoosmolality, resulting in lethargy, anorexia, nausea, vomiting, muscle cramps, coma, convulsions, and death.

Thromboembolism: obstruction of a blood vessel with thrombotic material carried by blood at the site of origin to the site of obstruction.

Tricyclic antidepressants (TCAs): the original antidepressants, with activity on a number of monoamine systems.

Tryptophan Hydroxylase (TPH): an enzyme that catalyses hydroxylation of L-tryptophan.

Volume of distribution (V_d): Artificial volume in which a drug distributes throughout the body, expressed as total amount of drug in the body (absorbed dose) in L divided by kg body weight.

INTRODUCTION

1.1. Prologue

The extent to which new and existing drugs interact with one or more neurotransmitter systems has become a subject of great interest to pharmacologists and toxicologists alike. Understanding the roles the monoamines serotonin, dopamine, noradrenaline, and gamma aminobutyric acid (GABA) play in regulating emotions and behaviours has led to the development of drugs which target particular modes of action of one or more of these monoamines. This has led to a deeper understanding of the mechanisms of the development of side effects and toxicity associated with these drugs. Not surprisingly, the vast majority of drugs that affect the monoamines have psychiatric applications.

Psychiatrists have come to commonly acknowledge that polypharmacotherapy, the practice of prescribing more than one drug, can prove successful in treating depression in otherwise refractory patients. There is some evidence to support this theory (Apter et al, 1994; Frye et al, 2000). Depressed and schizophrenic subjects alike also tend to take other medications, either by prescription, to combat untoward side effects, or to feed addictions to drugs of abuse, or both. "Doctor-shopping", in which people go from doctor to doctor until they find one willing to prescribe desired drugs, has become popular in people with histories of drug abuse (Longo et al, 2000). This is especially troubling in light of the complex interactions which can occur between psychiatric drugs and drugs of abuse. As polypharmacotherapy and doctor-shopping become

more common, the number of fatalities involving one or more monoaminergic drug is increasing (Ananth and Johnson, 1992; Goldman, 2000).

Many commonly abused drugs, including amphetamine-like compounds, cocaine, benzodiazepines, cannabinoids, and opioids affect the same monoamines targeted by antidepressants and antipsychotics, so the effect of drugs prescribed to treat psychiatric disorders may vary depending on the combination of drugs present. The incidence of psychiatric drug-related deaths within the state of Victoria and the role of monoaminergic drugs in fatalities are the subject of this dissertation.

This chapter presents a review of the pharmacology and toxicology of monoaminergic drugs acting on selected receptor systems. Their relevance to this dissertation and the research plan are also discussed.

1.2. Physicochemical properties of neurotransmitters

1.2.1. Serotonin

1.2.1.1. Pharmacology of receptor subtypes

Serotonin is one of several neurotransmitters synthesized by the body to help regulate a number of physiological functions, although its role in controlling mood is perhaps the most well-known.

Serotonin receptors can be found virtually everywhere in the body, which explains the widespread activity associated with this monoamine. To date, some 15 serotonin receptor subtypes have been identified, each linked with a different physiological role (see Table 1.1) (Veenstra-VanderWeele et al, 2000). The serotonin transporter protein (5-HTT), human tryptophan hydroxylase gene (TPH), vesicular monoamine transporter type-2 (VMAT2), and monoamine oxidase-A (MAOA) also play important roles in the regulation of intra- and extracellular serotonin levels. All receptor subtypes except 5-HT_{5B} and 5-HT_6 are currently recognized as having defined functions. The 5-HT_1, 5-HT_2, and 5-HT_{4-7} receptor families are members of the superfamily of G protein-coupled receptors with a predicted membrane topology composed of an extracellular N-terminal segment linked to an intracellular C terminus by seven transmembrane-spanning segments (Figure 1.1) (Ross, 1992). By contrast, the 5-HT_3 receptor is a ligand-gated ion channel that gates Na^+ and Ca^{2+} and has a predicted membrane topology akin to that of the nicotinic cholinergic receptor (Hall, 1992).
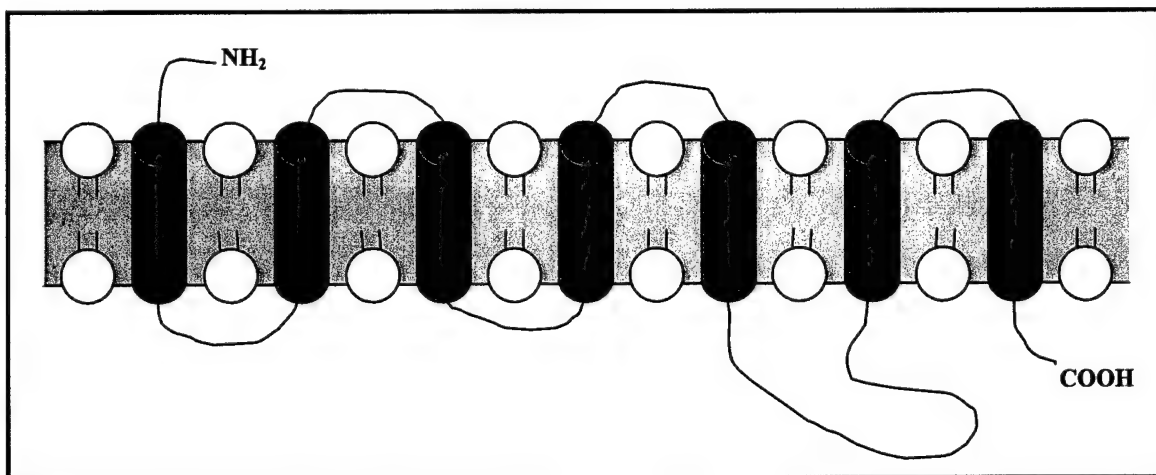
Figure 1.1. The 5-HT₁, 5-HT₂, and 5-HT₄₋₇ receptor subtypes are G protein coupled receptors, composed of a single subunit with seven presumptive transmembrane domains (after Ross) (Ross, 1992).

Table 1.1. Known serotonin receptor subtypes, location, and function (after Veenstra-VanderWeele et al)[1].

Subtype	Signal Transduction[2]	Location	Function/Notes[3]
5-HT$_{1A}$	- AC	Raphe nuclei, hippocampus	Knockout anxious, benzodiazepine-resistant
5-HT$_{1B}$ (=5-HT$_{1D\beta}$)	- AC	Subiculum, substantia nigra	Arterial dilation; Knockout aggressive, consumes more EtOH, cocaine
5-HT$_{1D}$ (= 5-HT$_{1D\alpha}$)	- AC	Cranial blood vessels	Arterial dilation; Pseudogene disrupted by Alu sequence
5-HT$_{1E}$ (= S31)	- AC	Cortex, striatum	--
5-HT$_{1F}$ (= 5-HT$_{1E}$= MR77)	- AC	Brain & periphery	--
5-HT$_{2A}$ (= 5-HT$_2$) D receptor	+ PLC	Platelets, smooth muscle, cerebral cortex	Platelet aggregation, contraction; neuronal excitation
5-HT$_{2B}$ (= 5-HT$_{2F}$)	+ PLC	Stomach fundus	Contraction
5-HT$_{2C}$ (=5-HT$_{1C}$)	+ PLC	Choroid plexus	Knockout obese, spontaneous fatal seizures
5-HT$_{3A}$ M receptor	Ligand-operated Na$^+$/Ca$^+$ channel	Peripheral nerves	Splice variants; gut motility, neuronal excitation
5-HT$_{3B}$ M receptor	Ligand-operated Na$^+$/Ca^{2+} channel	Peripheral nerves	Forms heteromer with 5-HT$_{3A}$
5-HT$_4$	+ AC	Hippocampus	2 splice variants: 5-HT$_{4I}$, 5-HT$_{4S}$; Neuronal excitation, atrial arrhythmias, gut motility
5-HT$_{5A}$ (= REC17)	Unknown	Gastrointestinal tract	Knockout explores more, LSD-insensitive
5-HT$_{5b}$	Unknown	Hippocampus	Unknown
5-HT$_6$ (= St-B17)	+ AC	Striatum	Unknown
5-HT$_7$	+ AC	Hypothalamus, intestine	80-90% homologous pseudogene; gut motility

[1] (Veenstra-VanderWeele et al, 2000); [2] AC: adenylyl cyclase; PLC: phospholipase C; [3] Knockout = receptor subtype not expressed.

Serotonin is synthesized *in vivo* from L-tryptophan (Figure 1.2) and degraded via metabolism to 5-hydroxyindole-3-acetic acid (5-HIAA) in a two-step process. The enzyme MAO-A is responsible for degrading 5-HT to 5-hydroxyindole acetaldehyde, which is then converted to 5-HIAA, via aldehyde dehydrogenase, and 5-hydroxytryptophol, via aldehyde reductase. By far, 5-HIAA is the major breakdown product of the two (Gillis, 1985). Using a separate metabolic pathway, some 5-HT is also eventually converted to melatonin.
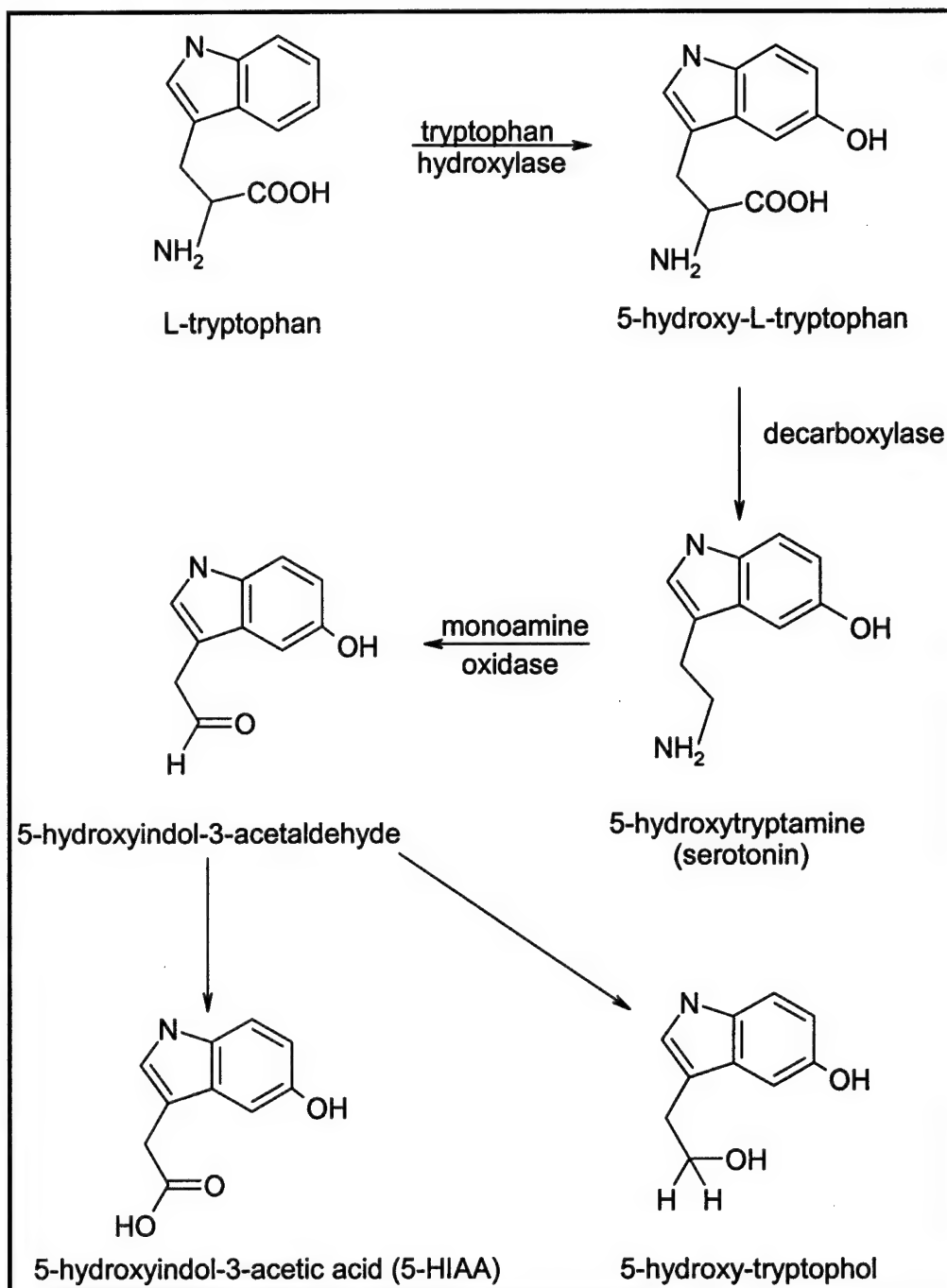
Figure 1.2. Synthesis and metabolism of serotonin (Gillis, 1985).

1.2.1.2. Physiological effects of serotonin

The role of serotonin (5-HT) in physiology is perhaps the most complex of the biogenic amines. As a neurotransmitter, 5-HT acts centrally, and has been implicated in causing depression, anxiety disorders, obsessive-compulsive disorder, psychosis, eating disorders, and substance abuse and dependence (Lucki, 1998). Additionally, it affects cognition, sleep, motor systems, appetite, sexual behaviour, temperature regulation and hormone secretion. There are a number of well-documented and replicated observations of altered 5-HT neurochemistry, including elevated whole blood 5-HT in autism (Cook and Leventhal, 1996) and low cerebrospinal fluid levels of 5-HT metabolite 5-hydroxyindoleacetic acid in violent suicide or impulsive aggression (Linnoila and Virkkunen, 1992).

Serotonin also plays important roles as a neuromodulator. Peripherally, serotonin is produced in enterochromaffin cells in the intestine, where it acts as a modulator of smooth muscle function in the cardiovascular and gastrointestinal systems. Free 5-HT is taken up and stored by platelets, and its release during platelet activation contributes to haemostatic processes. Serotonin can cause both vasoconstrictive and vasodilatatory effects via vascular 5-HT receptors (Frishman and Grewall, 2000). Additionally, activation of atrial 5-HT$_4$ receptors appears to be important in cardiac arrhythmias (Laer et al, 1998; Pino et al, 1998). The role of 5-HT (specifically the 5-HT$_3$ and 5-HT$_4$ receptors) in regulating gut motility has led to the development of drugs for the treatment of irritable bowel syndrome (Sanger, 1996).

1.2.2. *Dopamine*

1.2.2.1. Dopamine receptor pharmacology

Like those for serotonin, multiple receptors have been characterized for dopamine, all of which are G protein-coupled. At present, five distinct dopamine receptors are known to exist (Jarvie and Caron, 1993). Each dopamine receptor has a distinct anatomical distribution, as shown in Table 1.2. These are divided into two groups on the basis of their pharmacological and structural properties. The D1 class includes the D_1 and D_5 proteins, both of which have a long intracellular carboxy-terminal tail. These receptors stimulate formulation of cyclic AMP and phosphatidyl inositol hydrolysis. The D2 class includes the D_2, D_3, and D_4 receptors, which all have a large third intracellular loop. The D2 receptors decrease cyclic AMP formation and modulate K^+ and Ca^{2+} currents.

Table 1.2. Dopamine receptor distribution (after Jarvie and Caron)[1].

D_1 Receptor Family		D_2 Receptor Family		
D1	*D2*	*D2*	*D3*	*D4*
striatum	hippocampus	striatum	olfactory. tubercle	frontal cortex
neocortex	hypothalamus	SNpc2	nucleus accumbens	medulla
		pituitary	hypothalamus	midbrain

[1] (Jarvie and Caron, 1993) [2] SNpc: substantia negra pars compacta

Dopamine is synthesized *in vivo* in the terminals of dopaminergic neurons from tyrosine by the enzyme tyrosine hydroxylase, producing the intermediate L-dihydroxyphenylalanine (L-DOPA). L-DOPA is converted to dopamine by aromatic L-amino acid decarboxylase (AAD) (Figure 1.3.). Dopamine's actions are terminated by the sequential actions of the enzymes catechol-O-methyl-transferase (COMT) and MAO, or by reuptake of dopamine into the terminal.

1.2.2.2. Physiological effects of dopamine

Although originally regarded only as a precursor to noradrenaline, dopamine has come to be understood to have wide-ranging physiological effects and to distribute in tissues in a manner which is markedly different to noradrenaline. It is a central neurotransmitter particularly important in the regulation of movement, and is implicated in the symptoms of Parkinson's Disease (via D_2 receptors). At low concentrations, its primary interaction peripherally is with vascular D_1 receptors, leading to vasodilation. Additionally, low doses of dopamine cause increases in glomerular filtration rate, renal blood flow and Na^+ excretion. Because of these effects, dopamine is often used in the treatment of cardiogenic and hypovolemic shock (Higgins and Chernow, 1987). At slightly higher concentrations, dopamine exerts a positive inotropic effect on the myocardium by acting on β_1-adrenoceptors. It also causes the release of noradrenaline from nerve terminals, contributing to its cardiac effects. At much higher concentrations, however, it activates vascular α_1-adrenoceptors, leading to vasoconstriction. Thus, if dopamine is used in cases of life-threatening shock, blood pressure and renal function must be monitored closely (Higgins and Chernow, 1987).
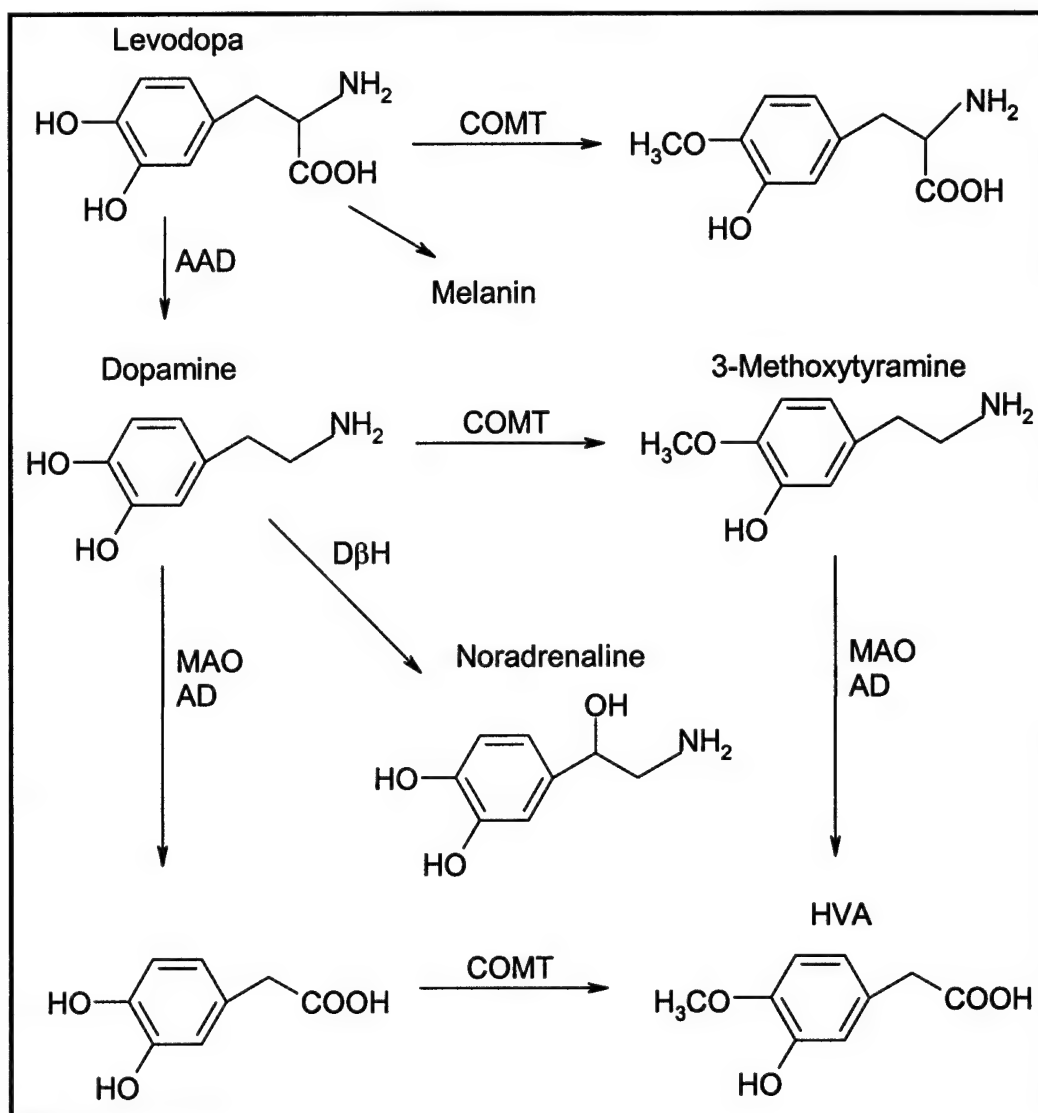
Figure 1.3. Synthesis and metabolism of dopamine. AAD = aromatic L-amino acid decarboxylase; AD = aldehyde dehydrogenase; COMT =catechol-O-methyl transferase; DβH = dopamine β-hydroxylase; MAO = monoamine oxidase.

In addition to Parkinson's disease, D_2 receptors have also been linked with the pathophysiology of schizophrenia (Seeman, 1987). The original, so-called "typical antipsychotics" bind non-specifically to dopamine receptors (both the D1 and D2 class of receptor proteins), which can

lead to the development of extrapyramidal side effects, including Parkinsonian-like movement disorders and tardive dyskinesia (O'Dell et al, 1990). Atypical antipsychotics, by contrast, bind only to the D2 class of receptors, with affinity in the nanomolar range. Typical antipsychotics include haloperidol, chlorpromazine, fluphenazine, and trifluoperazine. Risperidone, clozapine, olanzapine, thioridazine, and flupenthixol are all classed as atypical antipsychotics. Risperidone, clozapine, and thioridazine possess potent antiserotonergic (5-HT$_2$) as well as antidopaminergic (D$_2$) activity.

1.2.3. Noradrenaline

1.2.3.1. Noradrenaline receptor pharmacology

Two G protein-coupled adrenoceptors, α and β, have been characterised, each of which is known to have more than one subreceptor type (Ahlquist, 1948; Emorine et al, 1989; Granneman et al, 1993; Lands et al, 1967). To date, six distinct α- and three β-adrenoceptors have been cloned. Like dopamine, each noradrenaline receptor has a distinct anatomical distribution and function, as shown in Table 1.3.

Noradrenaline is converted from dopamine *in vivo* in mammalian postganglionic adrenergic nerves by dopamine β-hydroxylase (Figure 1.3). The metabolic pathways of noradrenaline and adrenaline are, however, more complex (Figure 1.4).

Table 1.3. Noradrenaline receptor distribution[1].

Subtype	Signal Transduction[2]	Location	Function/Notes
α₁ₐ	+ PL C, D, A₂	heart, liver, cerebellum, cerebral cortex, prostate, lung, vas deferens	contraction, glycogenolysis, gluconeogenesis, hyperpolarisation & relaxation
α₁ʙ	+ PL C, D, A₂	kidney, spleen, aorta, lung, cerebral cortex	contraction, glycogenolysis, gluconeogenesis, hyperpolarisation & relaxation
α₁ᴅ	+ PL C, D, A₂	aorta, cerebral cortex, prostate, hippocampus	contraction, glycogenolysis, gluconeogenesis, hyperpolarisation & relaxation
α₂ₐ	− AC; + PL C, A₂; + K⁺ channels; − Ca²⁺ channels	platelets, nerve terminals	platelet aggregation, decreased noradrenaline release
α₂ʙ	− AC; + PL C, A₂; + K⁺ channels; − Ca²⁺ channels	pancreatic islets, liver, kidney	decreased insulin secretion
α₂ᴄ	− AC; + PL C, A₂; + K⁺ channels; − Ca²⁺ channels	smooth muscles, cerebral cortex	contraction
β₁	+ AC, + L-type Ca²⁺ channels	heart, juxtaglomerular cells	increased force, rate of contraction & AV nodal conduction velocity, increased renin secretion
β₂	+ AC	smooth/skeletal muscle, liver	relaxation, glycogenolysis; uptake of K⁺, gluconeogenesis
β₃	+ AC	adipose tissue	lipolysis

[1] (Ahlquist, 1948; Emorine et al, 1989; Granneman et al, 1993; Lands et al, 1967) [2] AC: adenylyl cyclase; PL: phospholipase

The noradrenaline receptors are targeted and acted upon by a wide range of drugs. Drugs used in the treatment of hypotension or shock achieve their effect through activation of presynaptic α_1-adrenoceptors in vascular smooth muscle (Garcia-Sainz et al, 1985). Drugs used to treat hypertension, on the other hand, activate α_2-adrenoceptors in the cardiovascular control centres of the CNS, which suppress the outflow of sympathetic nervous system activity from the brain (Kobinger, 1978). Amphetamine-like compounds, including both street drugs and those used therapeutically as diet aids, to treat narcolepsy or attention-deficit disorder, also act on α- and/or β-adrenoceptors to varied effect. Some act as weak agonists, and most release endogenous noradrenaline and other monoamines (Mitler et al, 1993; Samanin and Garattini, 1993; Silverstone, 1986).

Non-selective β-adrenoreceptor agonists are mainly used to treat bronchoconstriction in asthma patients and as cardiac stimulants. Some of the major untoward side effects associated with β-agonists when used to treat asthma are caused by stimulation of β_1-adrenoceptors in the heart. Because of this, newer drugs with specific activity at β_2-adrenoceptors have been developed for asthma treatment (Kelly, 1985; Nelson, 1982).
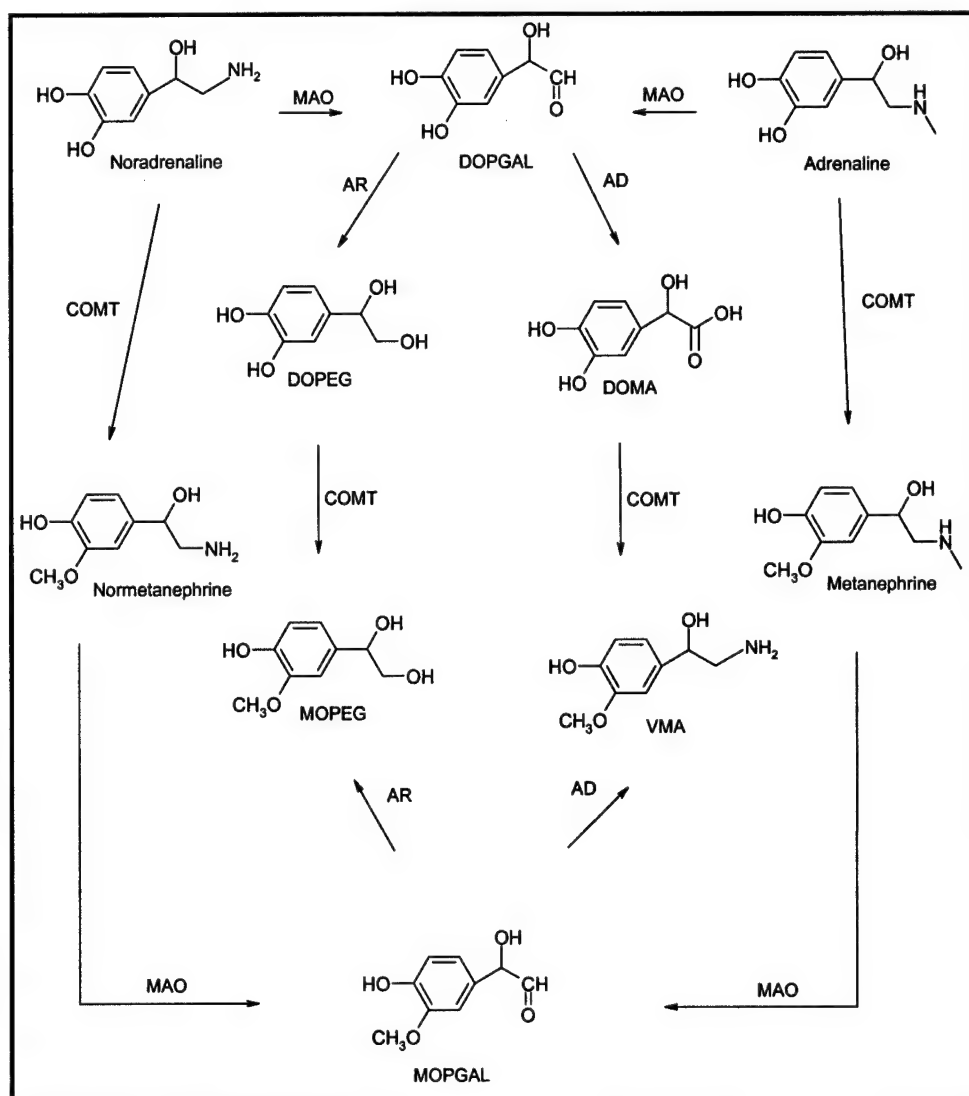
Figure 1.4. Metabolic breakdown of noradrenaline and adrenaline. MAO = monoamine oxidase; DOPGAL = 3,4-dihydroxyphenylglycoaldehyde; AD =aldehyde dehydrogenase; AR = aldehyde reductase; DOPEG = 3,4-dihydroxyphenylethylene glycol; DOMA = 3,4-dihydroxymandelic acid; COMT = catechol-O-methyltransferase; MOPEG = 3-methoxy-4-hydroxyphenylethylene glycol; VMA = 3-methoxy-4-hydroxymandelic acid; MOPGAL = 3-methoxy-4-hydroxyphenylglycoaldehyde (After Axelrod) (Axelrod, 1966).

1.2.3.2. Physiological effects of noradrenaline

Noradrenaline and adrenaline are similar structurally, and have similar pharmacological activity. They are approximately equal in their ability to stimulate β_1 adrenoceptors. Noradrenaline acts as a potent agonist at α-adrenoceptors but exhibits little activity at β_2-adrenoceptors. Adrenaline, however, is more potent than noradrenaline on the α- and β_2-adrenoceptors of most organs (Hartung, 1931). The immediate effects of low doses of noradrenaline in human beings are registered in the heart. Systolic and diastolic blood pressures and usually pulse are increased. Total peripheral resistance is raised, causing compensatory vagal reflex action to slow the heart. Peripheral vascular resistance increases in most vascular beds, while blood flow to the kidneys, liver, and skeletal muscle is reduced. Marked venoconstriction contributes to increased total peripheral resistance. Glomerular filtration rate is maintained unless the decrease in renal blood flow is marked. Noradrenaline constricts mesenteric vessels and reduces splanchnic and hepatic blood flows. Coronary flow is substantially increased. Unlike adrenaline, small doses of noradrenaline do not lead to vasodilation or lower blood pressure, as the blood vessels of skeletal muscle constrict rather than dilate (Black and Prichard, 1973; Bowman and Anden, 1981).

Other responses to noradrenaline are not prominent in humans. Hyperglycaemia and other metabolic effects attributed to adrenaline are generally seen only with high doses of noradrenaline. Intradermal doses of noradrenaline at sufficient doses causes sweating which cannot be blocked by atropine (Ogawa, 1976).

1.2.4.   Gamma aminobutyric acid (GABA)

1.2.4.1. GABA receptor pharmacology

GABA is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS), estimated to be involved at approximately one third of all synapses of the brain (Gaudrealt et al, 1991).   There are two main types of GABA receptors, the more prominent and well characterized of which is the $GABA_A$ receptor. The $GABA_A$ receptor is now understood to be a complex of several subunits. At the time of writing, six isoforms and 14 sub-types of $GABA_A$ had been identified (Sigel and Buhr, 1997). GABA itself predominantly binds to the α-subunit, while benzodiazepines bind to the β-subunit. Stimulation of the $GABA_A$ receptor opens the chloride ion channel and thereby increases conductance of Cl^- across the nerve cell membrane. This happens either by increasing the affinity of the $GABA_A$ receptor to GABA or by enhancing the coupling between the $GABA_A$ receptor and the chloride channel causing the channel to open (Figure 1.5) (Rang and Dale, 1991). Opening the ion channel reduces the potential difference between the inside and the outside of the cell and blocks the cell's ability to conduct nerve impulses (Gaudrealt et al, 1991).

The $GABA_A$ receptors are acted upon mainly by benzodiazepines, although other drugs have been shown to affect them as well. Benzodiazepines can bind to the β-subunit as full or partial agonists (eliciting a positive response), antagonists (blocking agonist action), or inverse agonists (eliciting a negative response).
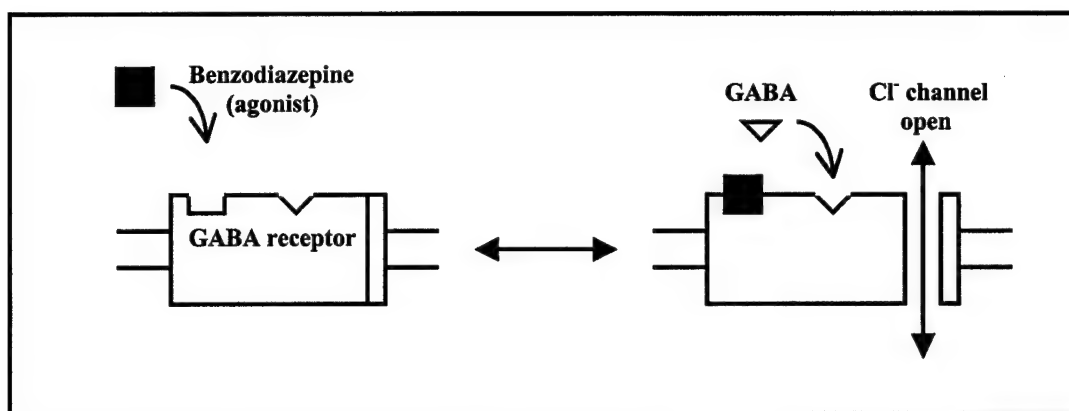
Figure 1.5. Model of benzodiazepine/GABA receptor interaction (from Rang and Dale, 1991). Benzodiazepines bind to GABA$_A$ receptor α-subunits. The consequent binding of GABA to a GABA$_A$ receptor β-subunit opens the chloride channel and reduces the potential difference between the inside and the outside of the cell.

The GABA$_B$ receptor is a G protein-coupled receptor, coupled both to biochemical pathways and to regulation of ion channels (Bonnano and Raiteri, 1993; Bowery, 1993). Nerves that transmit impulses via GABA are involved in many types of behaviour, and are linked to those modulated by opioids as well as drugs with serotonergic activity (Drummer, 2001).

1.2.4.2. Physiological effects of GABA

GABA acts as a CNS depressant (mainly in the cerebellum, cerebral cortex, hippocampus, and striatum) and is the principal inhibitory neurotransmitter in humans (Drummer, 2001). It mediates inhibition caused by local interneurons in the brain and possibly the presynaptic inhibition within the spinal cord. GABA thus has a wide range of effects and plays a role in the

pharmacological action of a wide variety of drugs. GABA's role in ion channel regulation (see section 1.2.4.1) has been exploited in anaesthetics, which act to increase the affinity of the $GABA_A$ receptor for GABA (Alifimoff and Miller, 1993). Additionally, it affects ganglionic neurotransmission, apparently by modulating the initial excitatory postsynaptic potential (EPSP) and inhibiting the K^+ conductance, or M current (Bowery et al, 2002; Ong and Kerr, 2000). Decreased transmission of GABA has also been shown to disrupt mammalian circadian rhythm (Kalsbeek et al, 2000) and to yield psychosis-related behavioural effects (Bast et al, 2001).
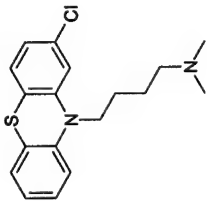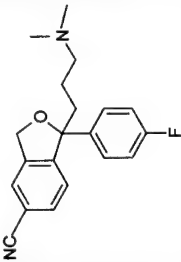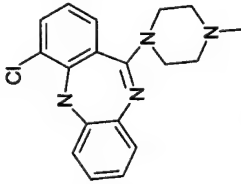
GABAergic transmission also plays a role in epilepsy, but its effects vary depending on the form of seizures and the functions of the GABAergic neurons in a given structure of the brain. Depaulis et al demonstrated that when GABA transmission had been globally increased, convulsive seizures were generally suppressed, whereas non-convulsive seizures were aggravated (Depaulis et al, 1997). If increased GABA transmission was localised to the substantia nigra, however, both convulsive and non-convulsive seizures were suppressed.
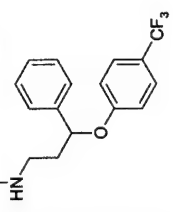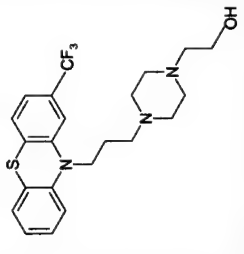
The manner in which GABA achieves its physiological effects is complex and occurs as a result of interaction with other neurotransmitters. For example, in schizophrenia, interaction between the serotonin $5-HT_{2A}$, and the muscarinic M_1 receptors has been theorised to be a potent modulator of GABA activity and, therefore, of $GABA_A$ receptors (Dean, 2001). In anxiety, $5-HT_{1A}$ receptors potentiate GABA activity (Nazar et al, 1999). Passive-avoidance behaviour appears to be linked to $GABA_A$, benzodiazepine, and dopamine D2 receptors (Dubrovina and Il'iuchenok, 2000). The role of 5-HT in modulating GABA activity is discussed further in section 1.3.4.3.
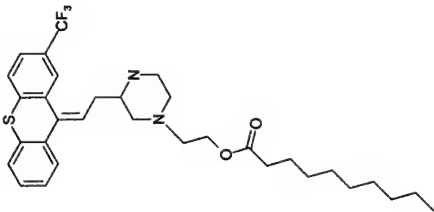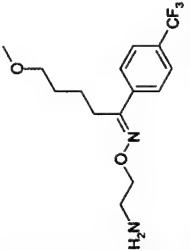
1.3. Pharmacology of monoamine-active drugs
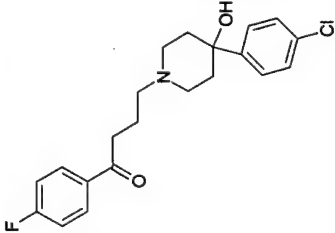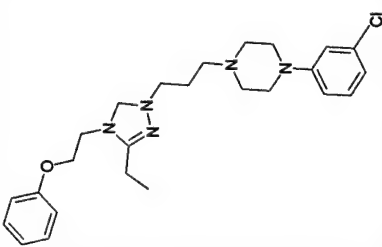
A large number of monoaminergic medications are basic and have pK_a's above 7.0. These compounds can broadly be classed in two groups: psychiatric drugs, including both antidepressants and antipsychotics, and drugs of abuse. Some commonalities in their physicochemical properties are observed (Table 1.4). They tend to have large volumes of distribution ($V_d \geq 3$ L/kg) making them susceptible to the phenomenon of postmortem redistribution, are metabolised to pharmacologically active metabolites, and affect primarily the dopaminergic and serotonergic neurotransmitter systems. Additionally, many of the psychiatric drugs (10 of the 16 studied in this work) have half-lives of the order of days rather than hours. A more detailed discussion of physicochemical and pharmacokinetic properties of each drug class studied follows.
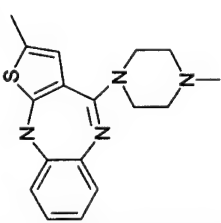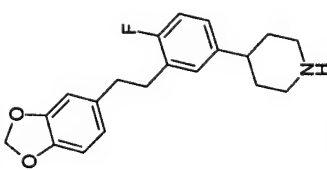
Table 1.4. Drug name, half-life, volume of distribution, metabolite, and neurotransmitter activity of psychiatric drugs studied[1].
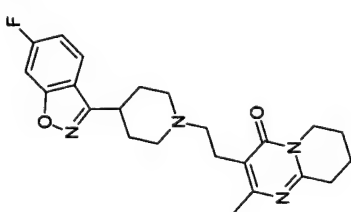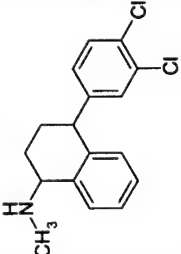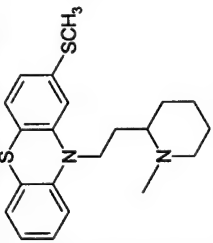
Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Chlorpromazine, pK$_a$=9.30	18-30	10-35, 0.98	chlorpromazine sulfoxide **norchlorpromazine** dinorchlorpromazine **7-hydroxy chlorpromazine** chlorpromazine N-oxide + conjugates	DA: ++ (D2) NA: α_1: ++ HIS: + (H$_3$)
Citalopram, pK$_a$ = 9.54	25-35	12-17, 0.80	**desmethyl citalopram** **didesmethyl citalopram** citalopram N-oxide propionic acid derivative	ST: (5-HT$_{1A/2A/3}$)
Clozapine, pK$_a$ = 1.92, 2.82, 7.65	4.5-7.5	5, 0.95	clozapine N-oxide **N-desmethyl clozapine**	DA: +++ (D1/D2/D4) MUSC: + (M$_1$) NA: ++ (α_1) ST: ++ (5-HT$_{2A/2C}$) HIS: + (H$_1$)
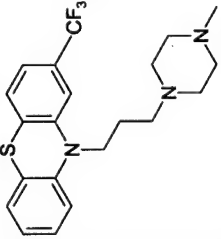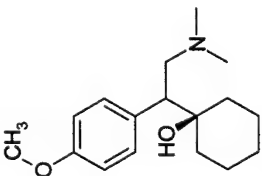
Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Fluoxetine, pK$_a$=9.73	24-72	26, 0.94	norfluoxetine	ST: +++ (5-HT$_{1A/1B/2A/\#C}$)
Fluphenazine, pK$_a$=3.9, 8.1	5-12 days	220, 0.99	**fluphenazine sulfoxide** **7-hydroxy fluphenazine** **fluphenazine N-oxide**	DA: + (D1)

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Flupenthixol decanoate, pK_a=2.69, 7.73	19-39	no data, >0.95	**flupenthixol sulfoxide** **flupenthixol N-oxide** + conjugates	DA: ++ (D1/D2)
Fluvoxamine, pK_a=9.05	16-26	25, 0.77	fluvoxamine acid	ST: + (5-HT$_{1A/1C/3}$)

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Haloperidol, pK_a=8.30	14-41	18-30, 0.90	4-fluorobenzoylpropionic acid 4-fluorobenzoylacetic acid **reduced haloperidol**	DA: +++ (D1/D2), ST: ++ (5-HT$_{2A/1C}$)
Nefazodone, pK_a=9.20	18	0.22-0.87, 0.99	**hydroxy nefazodone** **1-(*m*-chlorophenyl)-piperazine**	ST: + (5-HT$_{1A/1B,/2A\#C}$) NA: + α_1

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Olanzapine, pK_a=1.34	21-54	1000, 0.93	4'-N-desmethylolanzapine **10-N-glucuronide**	DA: + (D1/D2/D4), muscarinic: + (M_{1-5}), NA: ++ (α_1), ST: ++ (5-HT$_{2A/2C}$), HIS: + (H_1)
Paroxetine, pK_a=9.83	7-37	3-28, 0.95	methylene bridge lost resulting in catechol intermediate which is extensively sulphated & glucuronidated	ST: + (5-HT$_{1A/2A}$)

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Risperidone, pK_a=8.90	2.8	1.2, ~0.90	**9-hydroxyrisperidone**	DA: ++ (D2) ST: (5-HT$_{2A/2C}$)
Sertraline, pK_a=9.24	24-26	25, 0.99	**N-desmethylsertraline**	ST: + (5-HT$_{1A/1B/2A/\#C}$)
Thioridazine, pK_a=9.50	26-36	18, 0.96	**Mesoridazine** **N-desmethyl thioridazine** **Sulforidazine** thioridazine sulfoxide	DA: ++ (D1/D2) MUSC: ++ (M$_1$) ST: + (5-HT$_{2A}$) NA: + (α_1)

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Trifluoperazine, pKa=8.10	7-18	? , 0.91-0.99	trifluoperazine sulfoxide **7-hydroxytrifluoperazine** N-desmethyl trifluoperazine	DA: + (D1)
Venlafaxine pKa=9.24	3-7	4-12, 0.27	**O-desmethylvenlafaxine**	ST: ++(5-HT$_{1A}$) NA: + (α_1)

[1] (CompuDrug Inc., 1999)[Baselt, 2000 #1] [2] NA= noradrenaline; DA = dopamine; ST = serotonin; HIS: histamine, MUSC: muscarinic; + to +++ = active to strongly active.

1.3.1. Tricyclic antidepressants

The tricyclic antidepressants (TCAs) represent a class of drugs that show activity on a number of monoamine systems. While not studied in this research, they are introduced here to provide a means for comparison to the newer antidepressants and a basis for understanding drugs whose activity is specific for particular monoamines. TCAs generally affect reuptake of serotonin, noradrenaline, and dopamine in presynaptic neurons. This results in increases in available neurotransmitters, followed by plastic (formational) changes in pre- and postsynaptic receptors. TCAs have seen limited therapeutic use due to their association with wide-ranging physiological side effects. Such side effects include sedation, due to their affinity as agonists of histamine H_1 receptors, and vasodilation with orthostatic hypotension and reflex tachyarrhythmias, due to their effect on α_1-adrenoceptors. Also, a variety of anticholinergic side effects including dry mouth, blurred vision, urinary retention and constipation, amnesia, and cardiac arrhythmias arise as a result of their affinity at cholinergic muscarinic M_1 receptors. Furthermore, tertiary amine TCAs are demethylated to active metabolites which are generally potent inhibitors of noradrenaline uptake, thereby adding to the cardiovascular and CNS toxicity of the drug. Despite their side effect profile, a lack of significant response in up to 20-30% of patients, and narrow therapeutic ranges, TCAs remain effective in the treatment of refractory and severe depressive illness (Gessel, 1995; Sambunaris, 1997).

In an effort to decrease the occurrence of adverse drug reactions associated with the use of antidepressants, pharmaceutical companies have designed new drugs with higher specificity of action. In the early 1970s, it was recognized that serotonin played a major role in mood

regulation. It was therefore believed that drugs which selectively affect serotonin levels would be both more successful at treating mood disorders and have fewer side effects.

1.3.2. Serotonergic antidepressants

Drugs altering serotonin activity have been developed in the last 15 years with particular emphasis on the treatment of mood disorders. Fluoxetine was the first "selective serotonin reuptake inhibitor" (SSRI), followed by sertraline, paroxetine, fluvoxamine, and citalopram. Venlafaxine is referred to as a serotonin and noradrenaline reuptake inhibitor (SNARI) as it inhibits noradrenaline as well as serotonin at high doses. Nefazodone is an atypical antidepressant often referred to as serotonin antagonist reuptake inhibitor (SARI), which combines a powerful antagonism of $5\text{-}HT_{2A}$ receptors and some noradrenaline reuptake blockade (Goeringer et al, 2000b).

SSRIs differ from the TCAs in that their active metabolites are also serotonin reuptake inhibitors. In addition to depression, SSRIs have also been used successfully in the treatment of bulimia nervosa, obsessive-compulsive disorder, anxiety, and migraines.

The SSRIs have structural differences, but they do share some common features (Table 1.4). All SSRIs have a benzene ring with a halogen-containing functional group para to the rest of the molecule. In fluoxetine and fluvoxamine, this is a trifluoromethyl group, whereas paroxetine and sertraline have only a halogen (fluorine and chlorine, respectively). By contrast, the atypical antidepressant nefazodone and its congener, trazodone, each possesses a chlorine atom in the

meta position relative to the rest of the molecule. Venlafaxine retains the para-substituted moiety, but in its structure a methyl ester takes the place of the halogen group (see Table 1.4).

The V_d's of all antidepressants included in this research except nefazodone are above 3.0 l/kg, meaning all may be susceptible to postmortem redistribution (see section 1.7). The antidepressants included in this dissertation are fairly well absorbed after oral administration (see Table 1.5). With the notable exception of venlafaxine, they are strongly bound to plasma proteins and other cellular constituents (Table 1.4). These drugs all possess pK_a's between 8.5-10 and are all quite lipophilic, requiring biotransformation for elimination. Generally speaking, the highest pK_a values belong to the SSRIs, while the atypical antidepressants nefazodone and venlafaxine both possess pK_a's around 9.2. Plasma protein binding of fluoxetine, paroxetine, and sertraline is ≥95%, whereas the values for fluvoxamine and citalopram are much lower (77% and 50%, respectively) (van Harten, 1993). Venlafaxine experiences much less protein binding (27%) than the SSRIs as a class. The protein binding of nefazodone is similar to the SSRIs (99%).

Fluoxetine and citalopram are available as racemic mixtures. The isomers of fluoxetine are approximately equipotent, while citalopram's (+)-enantiomer possesses most of its serotonergic activity (Boegesoe and Perregaard, 1989; Wong et al, 1985). Paroxetine and sertraline both have 2 chiral carbon atoms, and each drug is used clinically as pure isomers. Fluvoxamine is the only available SSRI without optical isomers (van Harten, 1993). The enantiomers of both venlafaxine and nefazodone are essentially equal in activity in humans (Caccia, 1998).

Table 1.5. Summary of pharmacokinetic parameters for selected psychiatric drugs[1].

Drug	Recommended Daily Dose (mg)	Oral Bioavail. (%)	Plasma Conc. (μg/L)[2]	Route of Excretion (%)	% Excr Unchanged in Urine
Citalopram	20-60	80	50-600	renal: 15	12
Clozapine	25-450	43-67	60-1000	renal: 50, faeces 30	≤20
Chlorpromazine	25-2400	13-51	1-750	renal: 23	<1
Fluoxetine	20-80	>60	15-450	renal: 80, faeces:15	11
Flupenthixol	oral: 3-6/day; i.m.:20-40/2-3 wks	~40	>2	no reports	no reports
Fluphenazine	2.5-20 or 12.5-100/1-3 wks	2.7-3.4	0.3-23	monkeys - renal: 12-19 faeces: 56-69	<1
Fluvoxamine	100-300	53	88-550	renal: 94	2
Haloperidol	100+	42-78	0.5-250	no reports	<1
Nefazodone	100-300	20	0.53-1800	renal: 55, faeces: 20-30	<1
Olanzapine	5-20	35-70	4.1-26	renal: 57, faeces: 30	7
Paroxetine	20-50	30-60	0.8-62	renal: 64, faeces: 36	<2
Risperidone	2-8	38-94	11-240	renal: 70, faeces: 14	4-30
Sertraline	50-200	100	9.5-310	renal: 40-45, faeces: 44	<0.2

Drug	Daily Dose (mg)	Oral Bioavail. (%)	Plasma Conc. (µg/L)	Route of Excretion (%)	% Excr Unchanged in Urine
Thioridazine	100-800	>40	50-3900	renal: 34,faeces: 50	4
Trifluoperazine	2-40	81-100	0.5-4.0	rats – renal: 6 faeces: 91	0.3-1.0
Venlafaxine	75-150	12-45	70	renal: 87	5

[1] (Greene and Barbhaiya, 1997; He and Richardson, 1995; Holley et al, 1983; Jann et al, 1985; Jann, 1991; Joffe et al, 1998; Jorgensen et al, 1982; Koytchev et al, 1994; Mattiuz et al, 1997; Troy et al, 1997; van Harten, 1995). [2] Usual plasma concentrations following therapeutic use.

The metabolites of all SSRIs except fluvoxamine are active (Overmars et al, 1983), although those of paroxetine, sertraline and citalopram are not believed to contribute significantly to the overall clinical effect profile of the parent drugs. By contrast, norfluoxetine, which is approximately equipotent with fluoxetine and achieves similar blood concentrations, is likely to have a major contribution to the parent drug's efficacy and side effect profile (van Harten, 1993).

Most antidepressants included in this research are fairly well absorbed, but many also undergo extensive first-pass metabolism, resulting in low absolute bioavailabilities (range 12-100). The bioavailability of nefazodone, for example, is only 20%, and that of paroxetine can be as low as 30% (Table 1.5) (Baselt and Cravey, 2000; Greene and Barbhaiya, 1997; Joffe et al, 1998; Troy et al, 1994; Troy et al, 1997; van Harten, 1995).

Generally, elimination occurs over an extended period. The N-demethylated derivatives of SSRIs have terminal elimination half-lives approximately twice as long as their parent drugs (van Harten, 1993). For example, whereas the half-life of fluoxetine is about 50 hours, that of norfluoxetine extends the overall half-life of the drug to values between 150-200 hours, requiring several weeks for elimination. On the other end of the scale is the SNARI venlafaxine (half-life approximately 6 hours), while that of nefazodone is about 18 hours (Hardman and Limbird, 1996). Like the SSRIs, venlafaxine's active metabolite, O-desmethylvenlafaxine, has a half-life about twice as long as that of its parent drug (about 12 hours). The half-life of hydroxynefazodone is substantially shorter than that of nefazodone (about 3 hours). The half-lives of SSRIs are affected by autoinhibition in that they inhibit their own metabolism (Beno, 1996). Fluoxetine, norfluoxetine, fluvoxamine, sertraline, and paroxetine are all known to

exhibit autoinhibition. Autoinhibition appears to be most potent with fluoxetine, as it occurs for both parent drug and metabolite.

1.3.3. Antipsychotics

The antipsychotics studied in this dissertation (Table 1.4) all have some dopaminergic activity. The so-called atypical antipsychotics, including clozapine, risperidone, and thioridazine, antagonize 5-HT activity at the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors on the postsynaptic neuron. They belong to four separate categories:

- phenothiazines (chlorpromazine, fluphenazine, trifluoperazine, thioridazine)
- piperazine-substituted thioxanthenes (flupenthixol)
- dibenzepines (clozapine and olanzapine)
- heterocyclics (haloperidol and risperidone)

A discussion of the pharmacology of each type follows.

Phenothiazines have a three-ring structure in which two benzene rings are linked by a heterocyclic ring (Figure 1.6). If the nitrogen at position 10 is replaced by a carbon atom with a double bond to the side chain, the compound is a thioxanthene. Substitution of an electron-withdrawing group at position 2 increases the efficacy of phenothiazines and other tricyclic congeners. The nature of the substituent at position 10 also influences pharmacological activity.
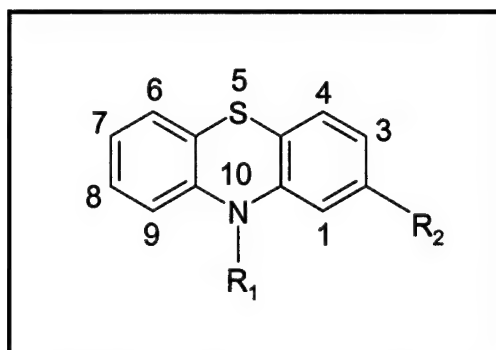
Figure 1.6. Generic phenothiazine structure.

Phenothiazines and thioxanthenes can be divided into three groups on the basis of substitution at this site: 1) those with an aliphatic side chain such as chlorpromazine, 2) those with a piperidine ring in the side chain, such as thioridazine, and 3) those with a piperazine group in the side chain such as fluphenazine and trifluoperazine (Figure 1.7). Group one compounds are low in potency but not clinical efficacy (Hardman and Limbird, 1996). Antipsychotics belonging to group two appear to be associated with fewer extrapyramidal side effects, possibly due to increased central antimuscarinic activity. Group three antipsychotics possess a high degree of potency. These compounds have weak anticholinergic activity, and carry a greater risk of inducing extrapyramidal side effects.
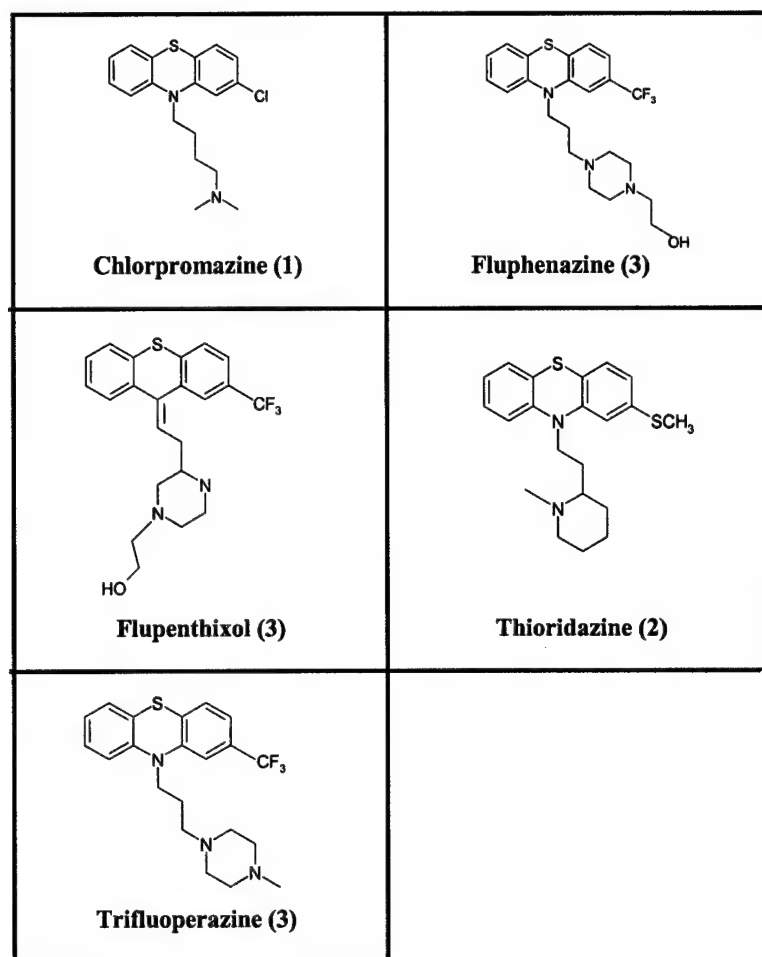
Figure 1.7. Phenothiazine and thioxanthene structures.

The dibenzepines, each containing a seven-member central ring, are a growing family of antipsychotic drugs (Figure 1.8). Clozapine and olanzapine both have an electronegative substituent at position 8, away from side-chain nitrogen atoms. They tend to be less potent than the other antipsychotics, have relatively low affinity at most dopamine receptors, and interact with muscarinic M_1, 5-$HT_{2(A/C)}$-serotonergic, α_1-adrenergic, and H_1 histaminic receptors.

Baldessarini and Frankenburg have reviewed their basic and clinical pharmacology in detail (Baldessarini and Frankenburg, 1991).
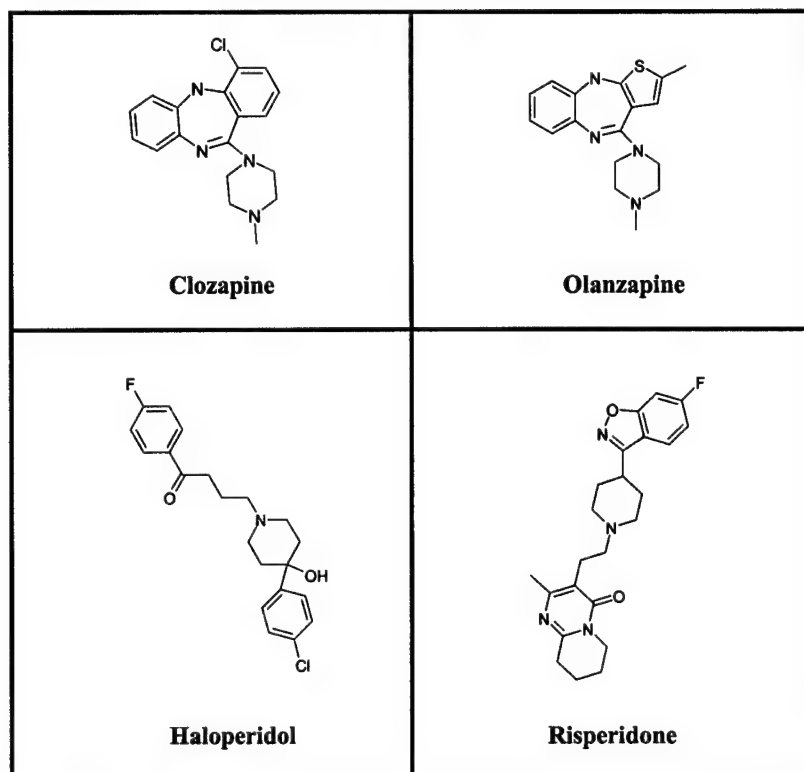
Figure 1.8. Chemical structures of heterocyclic neuroleptics.

Haloperidol (Figure 1.8) is a butyrophenone neuroleptic, and is quite potent and long acting. Another analogue is risperidone, a benzisoxazole derivative with prominent activity at $5\text{-}HT_2$ as well as D_2 receptors. It is considered a "quantitatively atypical" antipsychotic agent in that its extrapyramidal neurological side effects are limited if low doses are used, i.e. below 6 mg daily (Hardman and Limbird, 1996).

The pK_a's of the antipsychotic drugs included in this research span a much broader range than those of the antidepressants, (1.34-9.50). Several (clozapine, fluphenazine, flupenthixol, and olanzapine) have more than one ionization site, and therefore more than one pK_a value (Table 1.4). They are relatively lipophilic and, with the exception of risperidone (V_d = 1.2 L/kg), their V_d's are much higher than those for antidepressants, ranging from 5 L/kg for clozapine to 220 L/kg for fluphenazine. As with the antidepressants, antipsychotics are likely to undergo significant postmortem redistribution (see Section 1.7).

The optical isomers of most of these drugs are rarely separated in their marketed form, but in some cases their pharmacological activity differs between enantiomers. For example, the (-)-enantiomer of haloperidol is more pharmacologically active (Wu et al, 1999), while the (+)-enantiomer of risperidone is more active (Yasui-Furukori et al, 2001). The (+)-enantiomer of thioridazine is 4.1 times more potent than the (-)-enantiomer (Svendsen et al, 1988a).

Esterifying piperazine phenothiazines with long-chain fatty acids at a free hydroxyl group produces long-acting depot injections. These are slowly hydrolysed, producing long-acting effects lasting up to two weeks. They possess the same degree of pharmacological activity as the oral preparations, and are often used in delusional paranoid patients who believe their medicine is poison. Parenteral injections are sometimes preferred in highly agitated patients (Baldessarini, 1984). In Australia, the decanoate derivatives of fluphenazine and flupenthixol are registered as depot drugs.

After oral administration, some antipsychotic drugs have unpredictable patterns of absorption. For example, the oral bioavailability of chlorpromazine ranges from 13-51% (Table 1.5) (He and Richardson, 1995; Holley et al, 1983; Jann et al, 1985; Jann, 1991; Jorgensen et al, 1982; Koytchev et al, 1994; Koytchev et al, 1996; Mattiuz et al, 1997). Its absorption in the gastrointestinal tract is complicated by the presence of food and antacids in the stomach, which can alter physiological pH and therefore change the extent of first-pass metabolism (Yeung et al, 1993). It is possible that antiparkinsonian drugs with anticholinergic activity decrease absorption of antipsychotics when taken concomitantly (Simpson et al, 1980). However, parenteral administration of these drugs can increase neuroleptic bioavailability by up as much as a factor of 10, as first-pass metabolism is largely avoided.

Antipsychotics are highly membrane- and protein-bound, and accumulate in the brain, lung, and other highly perfused tissues (see Table 1.5). Terminal elimination half-lives typically range from 20 to 40 hours, and the metabolic pathways for some antipsychotics are complex, particularly with the butyrophenones and their congeners (Cohen et al, 1992).

Metabolism of antipsychotic drugs occurs via a number of pathways, including N-demethylation, oxidation, sulfoxidation, and hydroxylation (see Table 1.5). Like the serotonergic antidepressants, many of the target antipsychotics are metabolised into one or more compounds with significant pharmacological activity. Two exceptions are chlorpromazine, which is metabolised to nor- and di-norchlorpromazine (one-fourth and one eighth as active), and clozapine, whose two major metabolites norclozapine and clozapine N-oxide are not believed to possess any activity (Ackenheil, 1989).
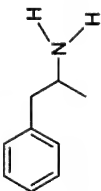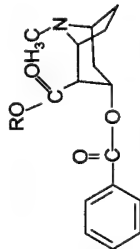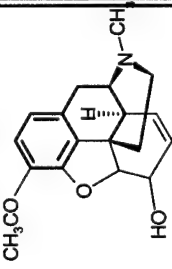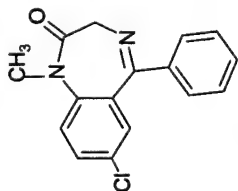
1.3.4. Drugs of abuse
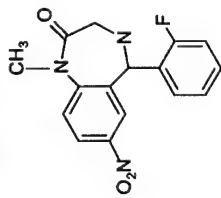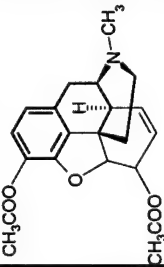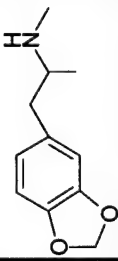
In forensic cases there are not only high incidences of abused drugs but often, in the same subjects there is also a relatively high incidence of drug treatment for psychiatric illnesses, including psychosis and depression. Commonly abused drugs include the amphetamines, benzodiazepines, cannabinoids, cocaine, and opioids. Blood concentrations of these drugs detected in the presence of psychiatric drugs in previous studies are discussed in section 1.5.4. All of these have varying affinity for the serotonin, dopamine, GABA, and/or noradrenaline receptor sites. A discussion of each group follows.
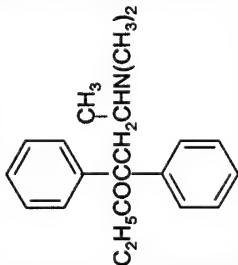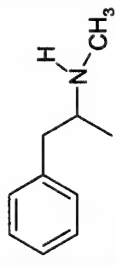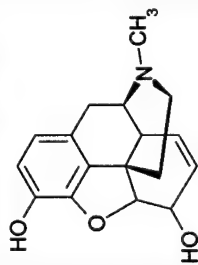
1.3.4.1. Amphetamines
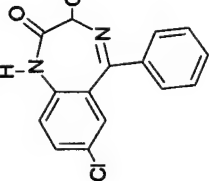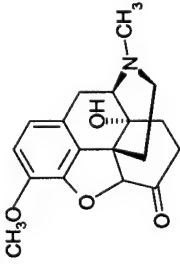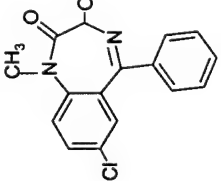
Compounds belonging to the amphetamine class of drugs have been studied in this dissertation and are shown in Table 1.6. The term "amphetamines" is commonly used to describe a group of compounds also known as the sympathomimetic amines, which consist of a benzene ring and an ethylamine side chain. A more accurate term, and the one which shall be used in this dissertation, is "amphetamine-like compounds". The amphetamine-like compounds are referred to as "sympathomimetic amines" because they act by either replacing monoamine neurotransmitters at, or facilitating release of real neurotransmitters from, nerve endings (Drummer, 2001). In replacing monoamines, amphetamine-like compounds act as false neurotransmitters, substituting for noradrenaline or dopamine, although such action occurs at higher concentrations and with less intensity than the endogenous neurotransmitters.
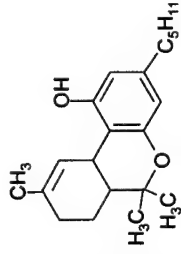
Table 1.6. Drug name, half-life, volume of distribution, metabolite, and neurotransmitter activity of drugs of abuse studied[1].

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Amphetamine, pKa = 9.90	7-34	3.2-5.6, 0.16	**p-hydroxyamphetamine** **norephedrine** **p-hydroxynorephedrine** phenylacetone	DA: ++ (D_1/D_2) ST: + (5-HT_2) NA: (α/β)
Cocaine, pKa = 8.60	0.7-1.5	1.6-2.7, 0.92	benzoylecgonine ecgonine methyl ester ecgonine **norcocaine**	DA: ++ (D_1/D_2) ST: + (5-HT_{1A}) NA: + (α/β)
Codeine, pKa = 8.20	1.9-3.9	3.5, 0.07-0.25	**morphine** **norcodeine** +conjugates	μ-opioid: + ; δ-opioid: ++ κ-opioid: + ST: + NA: + (α_2)-
Diazepam, pKa = 3.4	21-37	0.7-2.6, 0.96	**temazepam** **nordiazepam** **oxazepam** + conjugates	ST: + (5-HT_{1A}) GABA: ++ ($GABA_A$)

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Flunitrazepam, pKa = 1.80	20	4, ~0.80	7-NH$_2$-flunitrazepam 7-desmethyl flunitrazepam 3-hydroxy flunitrazepam + conjugates	ST: + (5-HT$_{1A}$) GABA: ++ (GABA$_A$)
Heroin, pKa = 7.60	1-1.5	25, 0.40	**6-acetylmorphine** **morphine** **normorphine** + conjugates	μ-opioid: + ; δ-opioid: +; κ-opioid: + ST: + NA: + (α$_2$)-
MDMA, pKa = 10.04	7.6	no data	**methylenedioxyamphetamine** mono- & di-hydroxy derivatives	DA: ++ (D$_1$/D$_2$) ST: + (5-HT$_2$) NA: (α/β)

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Methadone, pK$_a$ = 8.60 CH_3 $C_2H_5COCCH_2CHN(CH_3)_2$	15-55	4-5, 0.87	**methadol** **normethadol** EDDP EMDP	μ-opioid: +++ ; δ-opioid: + κ-opioid: + ST: + NA: + (α$_2$)-
Methamphetamine, pK$_a$ = 9.90 H N–CH$_3$	6-15	3.0-7.0, 0.10-0.20	**amphetamine** **p-hydroxy methamphetamine** **p-hydroxyamphetamine** + conjugates	DA: ++ (D$_1$/D$_2$) ST: + (5-HT$_2$) NA: (α/β)
Morphine, pK$_a$=8.1 N–CH$_3$	0.9-3.8	2-5, 0.35	**morphine-3-glucuronide** **morphine-6-glucuronide** normorphine	μ-opioid: +++ ; δ-opioid: + κ-opioid: + ST: + NA: + (α$_2$)-

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Oxazepam, pK_a = 1.70, 11.60	4-11	0.7-1.6, 0.87-0.94	oxazepam glucuronide	ST: + (5-HT$_{1A}$) GABA: ++ (GABA$_A$)
Oxycodone, pK_a = 8.50	4-6	1.8-3.7, no data	**oxymorphone** noroxycodone	μ-opioid: ++; δ-opioid: +++ κ-opioid: + ST: + NA: + ($α_2$)-
Temazepam, pK_a = 1.3	3-13	0.8-1.0, 0.97	**oxazepam** + conjugates	ST: + (5-HT$_{1A}$) GABA: ++ (GABA$_A$)

Drug	$T_{1/2}$ (hr)	V_d (L/kg), Fb	Metabolite (active metabolites in bold)	Activity[2]
Tetrahydrocannabinol, $pK_a=10.6$	20-57 (infreq. users) 3-13 days (frequent users)	4-14, 0.97	**11-hydroxy-THC** **8-β-hydroxy-THC** 8-α-OH-THC 8,11-dihydroxy-THC	CB_1: ++; CB_2: + DA: + (D_2) GABA: + ($GABA_A$)

[1] (CompuDrug Inc., 1999)[Baselt, 2000 #1]. [2] Abbreviations and symbols: NA= noradrenaline; DA = dopamine; ST = serotonin; HIS: histamine; + to +++ = active to strongly active.

Many direct-acting sympathomimetic drugs influence both α- and β-adrenoceptors and their subtypes, but the ratio of activities varies among drugs from predominantly α- to predominantly β- activity. The amphetamine-like compounds that lack hydroxyl groups on the ring and the β-hydroxyl group on the side chain act almost exclusively by causing the release of noradrenaline from adrenergic nerve terminals (Figure 1.9). Substitution of polar groups on the structure makes the resultant compound less lipophilic, so unsubstituted or alkyl-substituted compounds such as amphetamine and methamphetamine cross the blood-brain barrier more readily and have more central activity (Hardman and Limbird, 1996). Additionally, since both these drugs lack polar β-hydroxyl groups they have less direct sympathomimetic activity.
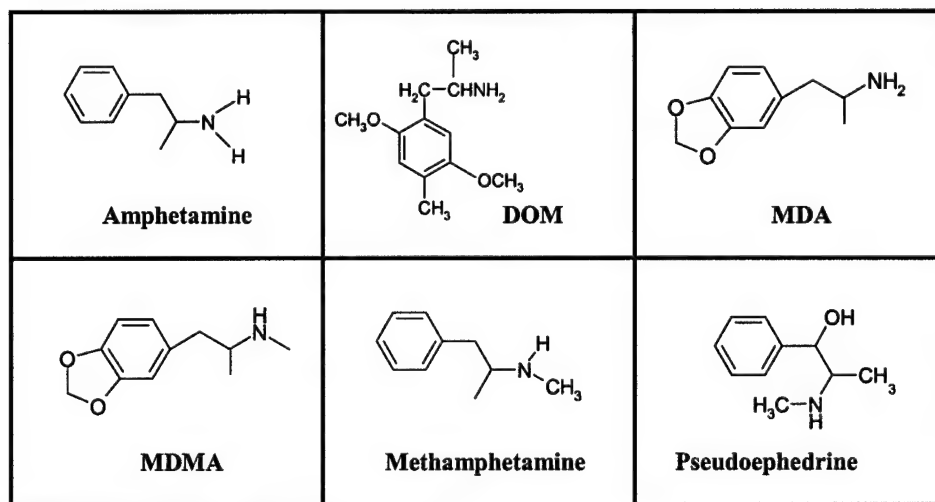
Figure 1.9. Chemical structures of amphetamine-like compounds.

Substitution on either the α- or β-carbon yields optical isomers. Laevorotatory substitution on the β-carbon confers greater peripheral activity, while dextrorotatory substitution on the α carbon

generally results in a more potent agonist. This means that *d*-amphetamine is more potent than *l*-amphetamine in central but not peripheral activity (Hardman and Limbird, 1996).

It has been demonstrated that each type of activity produced by phenalkylamine derivatives, including designer-amphetamines such as methylenedioxymethylamphetamine (MDMA) is associated with a distinct structure-activity relationship (Glennon, 1991). Certain phenalkylamines are more amphetamine-like in their effect, some are more DOM-like (4-methyl-2,5-dimethoxyamphetamine), and others produce an entirely different effect which is characterized by the effect caused by methylenedioxyamphetamine, or MDA (see Figure 1.9). The amphetamine-like effect (stimulation) appears to involve interaction with dopamine, whereas the DOM-like activity (relaxation) involves serotonin $5\text{-}HT_2$ receptors (Glennon, 1991). Most phenalkylamines, however, do not possess pure activity on a single neurotransmitter. MDMA, for example, produces amphetamine-like effects but not those associated with DOM.

Most amphetamine-like compounds undergo extensive biotransformation due to their lipophilicity. In contrast to the drugs discussed so far, amphetamines are not highly protein-bound (see Table 1.6). Their pK_a's are quite high (9.5-10.0) compared to other drugs of abuse. The d-isomers of amphetamines possess far more pharmacological activity and stimulant effect than the l-isomers, which are reported to have greater peripheral sympathomimetic activity (Baselt and Cravey, 2000). The V_d's of amphetamine-related compounds are generally above 3.0.

Pharmacokinetic properties of this class of compounds are shown in Table 1.7 (Baselt and Cravey, 2000; Helmlin et al, 1996; Vereby et al, 1988). The bioavailability of amphetamine-like compounds varies depending on the drug and route of administration. Amphetamine is effective after oral administration, and its effects last for several hours. The terminal half-life of *l*-amphetamine is 39 % longer than that of *d*-amphetamine, and the half-lives of both isomers are reduced to about 7 hours if the urine is acidified (Matin et al, 1977; Wan et al, 1978). Smoking methamphetamine vapours in a glass or aluminium pipe yields greater than 70 % of the drug, producing a rapid absorption and achieving of peak blood concentrations within minutes. By contrast, smoking methamphetamine normally (in a cigarette and presumably at lower temperatures) produces much lower yields (between 5-17 %). Amphetamine is produced in only small amounts following intravenous use or smoking (Cook, 1991).

Metabolism of amphetamine-like compounds involves a combination of hydroxylation (of the ring and the side chain carbon atom adjacent to it) and removal of the nitrogen (Ensslin et al, 1996; Helmlin et al, 1996). Drugs with alkyl groups attached to the nitrogen atom, such as methamphetamine and MDMA, are converted to other active amphetamines via the loss of the alkyl group. The methylenedioxy ring of MDMA is opened via hydroxylation. Amphetamine is largely inactivated during metabolism, being deaminated to phenylacetone, which is subsequently oxidized to benzoic acid and excreted as conjugates.

Table 1.7. Summary of the pharmacokinetics of selected drugs of abuse[1].

Drug	Daily Dose (mg)	Oral Bioavailability	Plasma Conc. (mg/L)	Principal Route of Excretion	% Excreted Unchanged in Urine
Amphetamine	5-15 abuse: up to 2000	5-70%[2]	0.04-0.11	renal: ~100%	1-74%[3]
Cocaine	10-120	~6-94%[2]	0.01-0.93	renal: ~100%	1-9%
Codeine	60-240	~100%	0.03-0.340	renal: 95%	?
Diazepam	2-40	~100%	0.15-4.0	renal:~53%	trace
Flunitrazepam	1-2	~64-77%	0.01-0.015	renal: ~90% faeces: <10%	<0.2%
Heroin	3-200	68% (rats)	0.02-0.30	renal: 45%	0.1%
Methadone	maintenance: 5-180	50%	0.03-1.06	renal: 8-75%	5-22%[3]
Methamphetamine	2.5-15	>70%	0.02-0.03	renal: ~50%	2-76%[3]
MDMA	100-150		0.11-0.30	renal: 72%	65%
Morphine	5+	10-50%	0.05-2.60	renal: 87%	10%
Oxazepam	30-60	93%	0.31-1.44	renal: 61%	trace
Oxycodone	15-30	80%	0.01-0.04	renal: 33-61%	13-19%
Temazepam	15-30	>80%	0.21-0.87	renal: 82%	1.5%
Δ^9-THC	5-20	22%	0.05-0.19	renal: 30% faeces: 40%	trace

[1] (Dahlstrom et al, 1979; Helmlin et al, 1993; Klem et al, 1986; Knowles and Ruelius, 1972; Locniskar and Greenblatt, 1990; Perez-Reyes et al, 1981; Renzi and Tam, 1979; Schwartz, 1979; Stanski et al, 1976; Vereby et al, 1988; Wretlind, 1977). [2] dependant on route of administration. [3] dependant upon urinary pH.

1.3.4.2. Cocaine

Cocaine was the first local anaesthetic, first introduced into clinical practice in 1884 for

ophthalmological surgery (Hardman and Limbird, 1996). However, because of its toxicity and

addictive properties, work began in 1892 to find safer synthetic substitutes. Cocaine is an ester of

benzoic acid and the complex alcohol 2-carbomethoxy, 3-hydroxy-tropane (Figure 1.10).
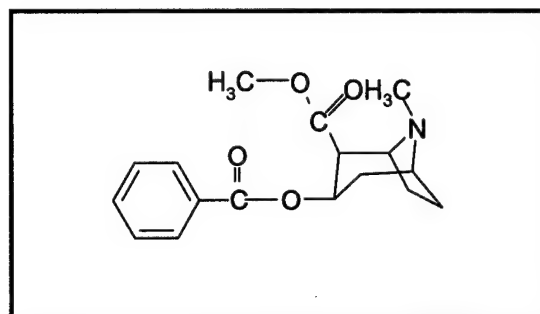
Figure 1.10. Chemical structure of cocaine.

The linking groups in cocaine determine some of the pharmacological properties of the

compound. For example, the ester link is hydrolysed readily by plasma esterases. The attached

benzene ring increases both the potency and duration of action due to enhanced partitioning of

the drug to tissues where it imparts pharmacological activity, and decreases the rate of

metabolism by plasma esterases and liver enzymes (Courtney and Strichartz, 1987).

Taking cocaine causes inhibition of the reuptake of released monoamines, which prolongs action

of the nerve impulse or disrupts proper transmission from occurring (Drummer, 2001).   Like the

other drugs of abuse already discussed, cocaine exerts specific effects on the monoamine

systems, particularly those of dopamine, noradrenaline, and serotonin. Chronic use of cocaine causes reductions in the noradrenaline and serotonin metabolites MHPG (3-methoxy-4-hydroxyphenethyleneglycol) and 5-HIAA (5-hydroxyindoleacetic acid) (Hardman and Limbird, 1996).

Cocaine is one of the most potent of the naturally occurring central nervous system stimulants. It is extremely water-soluble and is rapidly inactivated by the hydrolysis of one or both of the ester linkages. Its pK_a is comparable to those of opioids, and its V_d is within the range of values for the benzodiazepines included in this work (Table 1.6). Cocaine is 92 % protein bound, and there is a marked stereoselectivity in its psychomotor stimulant effects, with the (-)-enantiomer producing increases in locomotor activity and operant behaviour at doses 340 times lower than the highest doses of the (+)-enantiomer that were inactive (Katz et al, 1990).

Pharmacokinetic information about cocaine is included in Table 1.7. Intravenous injection, nasal insufflation, and smoking are all preferred over oral administration of cocaine, although oral use is becoming more popular among naïve users and women. Drug yield depends greatly on route of administration. Depending on the technique and temperature used, yields can approach 70 % (Cook, 1991). However, when smoking cocaine base with tobacco, yields are reduced to approximately 6 %. Snorted cocaine has a very high bioavailability (~94 %), but peak concentrations take much longer to achieve and are much lower than other routes (Drummer, 2001). The half-life of cocaine in plasma is about 50 minutes, but inhalant (crack) users typically desire more cocaine after 10 to 30 minutes.

Intranasal and intravenous use also result in a high of shorter duration than would be predicted by plasma cocaine concentrations, suggesting that declining tissue/brain concentrations are associated with termination of the high, leading to further cocaine use. The major route of cocaine metabolism involves hydrolysis of its two ester groups. Benzoylecgonine (BZE), produced upon loss of the methyl group, represents the major urinary metabolite and can be found in the urine for 2 to 5 days after use. Because of this, BZE tests are useful in detecting past cocaine use, as heavy users have been found to have detectable amounts of the metabolite in urine for up to 10 days following a binge. In blood or plasma, cocaine is hydrolysed to ecgonine methyl ester (EME) by cholinesterase, the reaction rate of which is dependent on temperature and drug concentration (Stewart et al, 1977). Formation of EME is thus an issue in the long-term storage of blood or plasma specimens containing cocaine as it may lead to inaccurate assessment of cocaine concentrations at time of death.

1.3.4.3. Benzodiazepines

Benzodiazepines are among the most commonly prescribed drugs worldwide, used mainly in the treatment of anxiety disorders and insomnia. The term benzodiazepine refers to the portion of the structure composed of a benzene ring (A) fused to a seven-membered diazepine ring (B) (Figure 1.11). Since all the important benzodiazepines contain a 5-aryl substituent (ring C) and a 1,4-diazepine ring, however, the term has come to mean the 5-aryl-1,4-benzodiazepines (Hardman and Limbird, 1996)
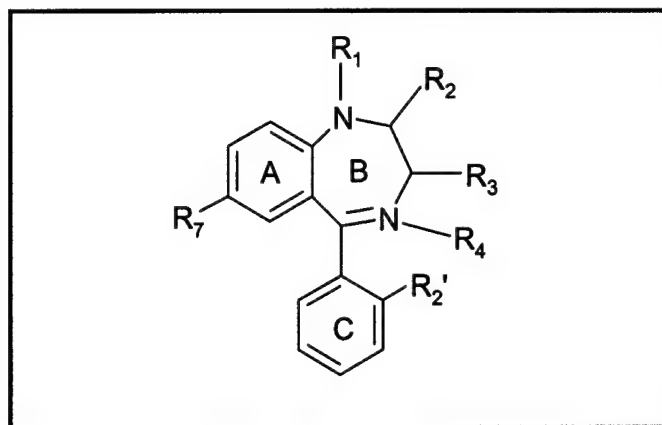
Figure 1.11. Generic benzodiazepine structure.

Diazepam, oxazepam, and temazepam are all considered traditional benzodiazepines (Figure

1.12). Temazepam is a metabolite of diazepam, and oxazepam is formed in the metabolism of

temazepam. The 7-nitrobenzodiazepines, such as flunitrazepam, share the basic structure of

benzodiazepines with the exception of a nitro-group in place of the chloride on the fused benzene

ring (A). Triazolam substitutes a triazole functional group in place of the methyl in position R_1,

whereas midazolam has an imidazolic group occupying R_1 and R_2 positions. The basicity of the

imidazole ring nitrogen on midazolam and of the triazole group on triazolam allows water-

soluble salts and well-tolerated injectable solutions to be prepared. The methyl groups on

midazolam's imidazole ring and triazolam's triazole group appear to be largely responsible for

their relatively rapid metabolism (elimination $t_{1/2}$ = 1-4 hours for both compounds) (Gerecke,

1983).
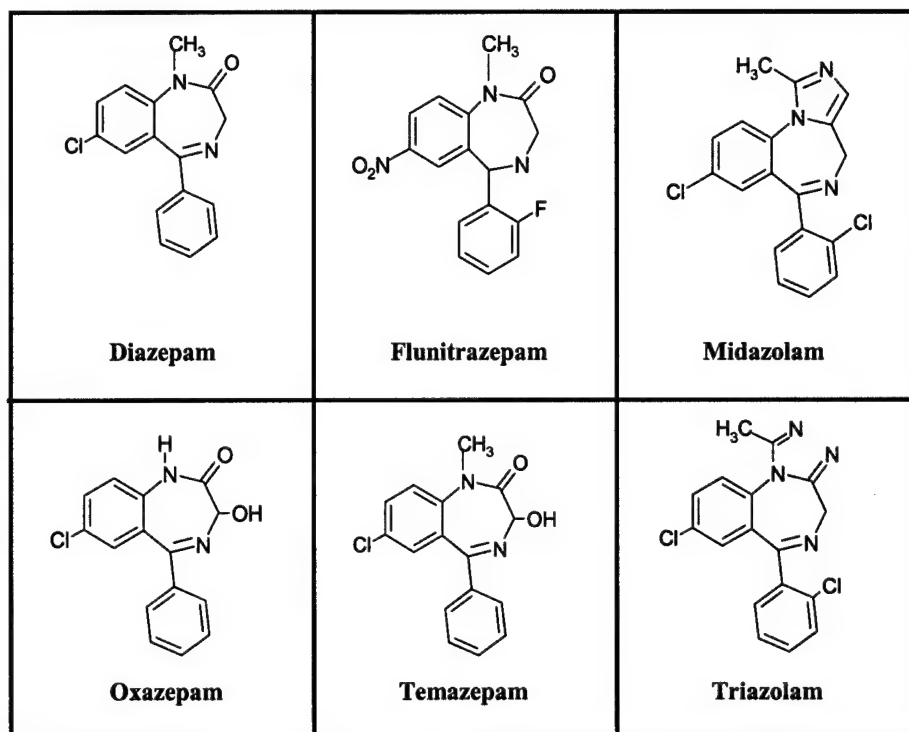
Figure 1.12. Chemical structures of selected benzodiazepines.

The lipophilicity of benzodiazepines varies more than 50-fold according to the polarity and electronegativity of the various substituents (Hardman and Limbird, 1996). The pK_a's of benzodiazepines are the lowest of all drugs of abuse studied in this dissertation, with the exception of oxazepam, which has a second ionisation site with a pK_a of 11.60 (Table 1.6). V_d's for these drugs are also comparatively low -- the highest value out of the target drugs belongs to flunitrazepam (4 L/kg). They are extensively protein bound, with values ranging from 0.80-0.97, and are well absorbed following oral administration. Table 1.7 shows pharmacokinetic parameters for benzodiazepines studied in this research (Baselt and Cravey, 2000; Klem et al, 1986; Knowles and Ruelius, 1972; Locniskar and Greenblatt, 1990; Wretlind, 1977). The initial and most rapid phase of benzodiazepine metabolism involves modification and/or removal of the

substituent. In most cases, the eventual products are biologically active N-desalkylated compounds. The second phase of metabolism involves hydroxylation at position 3 and often also usually yields an active derivative (such as oxazepam from nordiazepam). The third phase is conjugation of the 3-hydroxyl compounds, principally with glucuronic acid to form inactive products. The aromatic rings (A and C) of the benzodiazepines are hydroxylated to only a small extent. The only important metabolism at these sites is the reduction of the 7-nitro substituents of clonazepam, nitrazepam, and flunitrazepam, producing inactive amines with half-lives of between 20-40 hours (Hardman and Limbird, 1996).

1.3.4.4. Cannabinoids

The cannabis plant has been cultivated for centuries both for the production of hemp fibre and for its presumed medicinal and psychoactive properties. There are 61 different cannabinoids that have been identified in the smoke from burning cannabis. The chemical structures of Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^9-THC-carboxylic acid, a principal metabolite, are shown in Figure 1.13 (Perez-Reyes et al, 1981). Δ^9-THC produces most of the characteristic pharmacological effects of smoked marijuana.
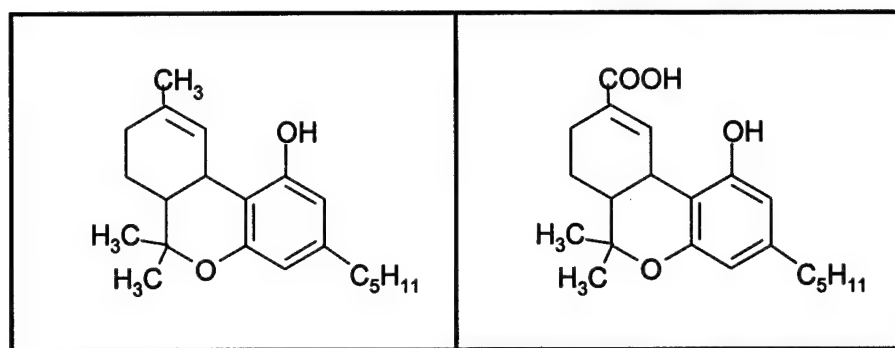
Figure 1.13. Chemical structure of a) Δ^9-tetrahydrocannabinol (THC) and b) Δ^9-THC-carboxylic acid.

Two G protein coupled-receptors for cannabinoids have been identified in brain (Devane et al, 1988) and have now been cloned (Matsuda et al, 1990). The CB_1 receptor and its splice variant CB_{1A} are found predominantly in the brain, with highest densities in the hippocampus, cerebellum, and striatum. The CB_2 receptor is found mainly in the spleen and in haemopoietic cells. Two putative endogenous substrates for brain neuronal CB_1 receptors have been identified: N-arachidonylethanolamine (commonly referred to as anandamide) and sn-2-arachidonylglycerol (Chaperon and Thiebot, 1999). Both are synthesized by neurons and brain tissue in response to increased intracellular calcium concentrations, and both are substrates for fatty acid amide hydrolase (Hillard, 2000). It has been theorised that these endogenous compounds play roles in the regulation of both gestation and of gastrointestinal motility (Hillard, 2000).

Δ^9-THC is highly lipophilic and undergoes extensive biotransformation prior to excretion. Its pK_a value of 10.6 is the highest of all drugs studied. It has a large V_d (4-14 L/kg) and is highly protein bound. See Table 1.6 for details for this drug.

Only about 22 % of the THC in a 'joint' can be recovered from smoking, during which the inactive compounds Δ^9-THC-carboxylic acid and cannabidiol are converted to THC (see Table 1.7) (Mikes and Waser, 1971). Peak plasma levels are not reached until 1-3 hours after oral ingestion of THC and are considerably lower than after smoking or intravenous injection, mainly due to significant first-pass metabolism. Absorption from the gut is believed to be 90-95 % complete (Hollister et al, 1981; Lemberger and Rubin, 1975).

The metabolic pathway of THC is complex and involves conversion to the active metabolites 11-OH-THC and 8-β-OH-THC, neither of which are measured at appreciable plasma concentrations and are unlikely to contribute significantly to acute effects except after oral use, in which 11-OH-THC activity is significant. Multiple inactive compounds are also formed either by further oxidation or conjugation. Many of these persist in the urine for up to several weeks after a single dose (Kanter and Hollister, 1977; Lemberger and Rubin, 1975; Wall et al, 1983).

1.3.4.5. Opioids

Drugs derived from opium are referred to as opioids and include morphine, codeine, and a wide variety of synthetic congeners derived from them and from thebaine, another component of opium. They are primarily used in the treatment of moderate to severe pain. Some of the CNS mechanisms that reduce the perception of pain, however, also produce a state of well-being and euphoria, leading to a high potential for abuse.

The principal mechanism of action of opioids is to interact with specific μ-, δ-, and κ-opioid receptor binding sites, particularly in the brain and spinal cord. Opioids are believed to mimic actions of endogenous compounds with natural analgesic properties, such as β-endorphin, leu-enkephalin, met-enkephalin, dynorphins A & B, and neoendorphins α and β (Drummer, 2001). These compounds are found at brain regions where the opioid receptors are located and are released by the body to reduce the sensation of pain. Each of the opioid receptors is associated with different pharmacological effects, which have been reviewed extensively by Drummer (Drummer, 2001). Briefly, the μ-opioid receptor is primarily responsible for opioid-induced analgesia; the δ-opioid receptor mediates analgesia, respiratory depression, euphoria and dependence; and the κ-opioid receptor mediates spinal analgesia and sedation, and is associated with only minor physical dependence.

Codeine is 3-O-methylmorphine, with the methyl substitution being on the phenolic hydroxyl group. Thebaine, which has little analgesic action but which is a precursor of the drugs oxycodone and naloxone, differs from morphine only in that both hydroxyl groups are methylated and that the ring has two double bonds. Molecular models show certain common characteristics, as seen in Figure 1.14. Among the important properties of this class of drugs are their affinity for various species of opioid receptors, their activity as agonists versus antagonists, their lipid solubility, and their resistance to metabolic breakdown. Blockade of the phenolic hydroxyl at position 3, as in codeine and heroin, drastically reduces binding to μ receptors. These compounds are converted to the potent analgesics morphine and 6-acetyl morphine, respectively, in vivo.
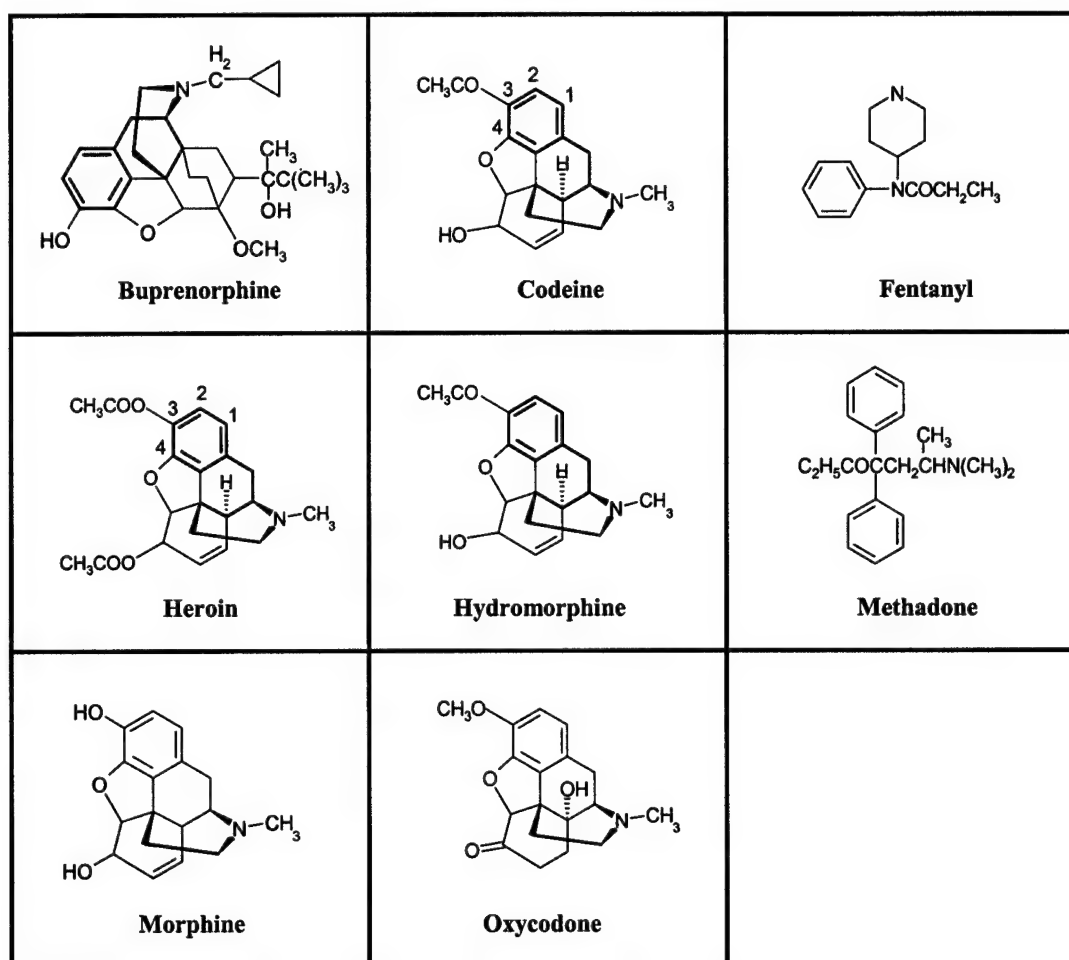
Figure 1.14. Chemical structures of selected opioids.

Like the other classes of drugs of abuse discussed this far, opioids exert specific effects on

noradrenaline, and serotonin. Specifically, morphine interacts with 5-HT-containing neurons in

the brain (Li et al, 2001; Mikkola et al, 2001) and adrenoceptors (Gadek-Michalska et al, 1997;

Gray et al, 1999; Hogger, 2000). The physiological effects of these interactions are discussed in

Section 1.5.3.5.

Opioids are only slightly basic; those studied in this research possess pK_a values ranging 7.60-8.60 (Table 1.7). Codeine, heroin, and methadone are relatively lipophilic, although morphine is not. When therapeutic concentrations of morphine are present in plasma, about one-third of the drug is protein bound, although methadone, used in narcotic maintenance treatment of former heroin addicts, is 87 % protein bound. Although used as a racemic mixture, (-)-methadone possesses the vast majority of pharmacological activity (Sullivan et al, 1972). Interestingly, only the (-)-enantiomer of morphine is formed naturally (Stringer et al, 2000). The V_d's of most opioids (with the exception of heroin) are fairly low.

Most opioids, including hydromorphone, methadone, morphine, and oxycodone, are readily absorbed from the gastrointestinal tract (Bouer et al, 1999; Mandema et al, 1996). Because of this, morphine and hydromorphone are also available as suppositories. The more lipophilic opioids, such as buprenorphine, fentanyl, and methadone, are readily absorbed through the nasal or buccal mucosa (Weinberg et al, 1988). The most lipophilic (fentanyl and methadone) can also be absorbed transdermally (Portenoy et al, 1993). Opioids are absorbed readily after subcutaneous or intramuscular injection and can adequately penetrate the spinal cord following epidural or intrathecal administration (Dahlstrom et al, 1979; Renzi and Tam, 1979; Stanski et al, 1976). With the exception of methadone, opioids have low oral bioavailability than after parenteral administration due to variable but significant first-pass metabolism in the liver. Although the primary site of action of morphine is the CNS, in the adult only small quantities pass the blood-brain barrier, and at a much slower rate than other, more lipid-soluble opioids such as codeine, heroin, and methadone (Hardman and Limbird, 1996).

The major pathway for the metabolism of morphine is conjugation to form both active and inactive glucuronidated metabolites. The pharmacological activity of morphine-6-glucuronide (M6G) is higher than that of morphine. M6G given systemically is approximately twice as potent as morphine, but when the blood-brain barrier is bypassed intracerebrally, M6G is approximately 100-fold more potent than morphine (Paul et al, 1989). Little morphine is excreted unchanged. It is eliminated largely by glomerular filtration, primarily as morphine-3-glucuronide; 90 % of the total excretion takes place during the first 24 hours (Table 1.7). Enterohepatic circulation of morphine and the glucuronides occurs, which accounts for the presence of small concentrations of morphine in the faeces and urine several days after the last dose (Hardman and Limbird, 1996).

1.3.4.6. Summary of drug of abuse neurotransmitter activity

A summary of the manner in which the drugs of abuse discussed in this section interact with the neurotransmitters dopamine, GABA, noradrenaline, and serotonin is shown in Table 1.8. Where such knowledge has been established, the specific subreceptor involved with particular functions is also listed.

Table 1.8. Neurotransmitter activities of selected drug types[1].

Drug Class	Neurotransmitter Receptor Activity/Function[2]
Amphetamines	brain DA: stimulation brain 5-HT: relaxation (5-HT$_2$); long-lasting or permanent neurotoxicity (especially from MDMA & methamphetamine)
Benzodiazepines	brain 5-HT: modulation of GABA$_A$/benzodiazepine receptor complex; enhances benzodiazepine anxiolytic effect (5-HT$_{1A, 3}$)
Cannabinoids	brain DA: reinforcement of self-administration, psychotogenic effect (D$_2$) brain 5-HT: inhibition of serotonin release by CB$_1$ brain NA: inhibition of noradrenaline release by CB$_1$ brain GABA: antianxiety effect (GABA$_A$/benzodiazepine receptors)
Cocaine	brain DA: reinforcement of self-administration (D$_2$) brain 5-HT: modulation of mood-effect
Opioids	brain DA, 5-HT: acute locomotor stimulation by morphine spinal 5-HT: development of morphine tolerance plasma NA: opioid-induced vertigo

[1] (Ameri, 1999; Freund et al, 1990; Gadek-Michalska et al, 1997; Gray et al, 1999; Halasy et al, 1992; Hogger, 2000; Katona et al, 2001; Li et al, 2001; Madras et al, 1989; Mikkola et al, 2001; Molliver et al, 1990; Nakazi et al, 2000; Nazar et al, 1999; Ritz et al, 1987; Soderpalm and Engel, 1989, 1990, 1991; Soderpalm et al, 1997; Volfe et al, 1985; Voruganti et al, 2001; Walsh et al, 1994) [2] Affected subreceptor shown in parentheses, if known.

1.4. Isoenzyme metabolism

Some of the drugs included in this study share common features with regard to enzyme

metabolism. Many of these drugs are inhibitors and/or substrates for the cytochrome P450

(CYP450) isoenzymes (Table 1.9). CYP450's are considered to be the most important family of

enzymes involved with phase I metabolism of drugs and other substances. Localised in the

endoplasmic reticulum, mitochondria, and nuclear and plasma membranes, the P450's are haeme

proteins usually in the ferric (Fe^{3+}) state (Moody, 1996). When reduced to the ferrous (Fe^{2+})

state, they bind to O_2 or CO. The most common P450 reaction is monooxygenation (see Figure

1.15). In this cyclic reaction, one oxygen atom is incorporated into the substrate while the other

is reduced to water (Moody, 1996). At the time of writing, there are 481 cytochrome P450 genes

which have been discovered, although only 221 have been observed in mammals. Of these, only

11 have been found to be involved with metabolism of xenobiotics in humans, represented in the

following subfamilies: 1A (1 gene), 2A (1), 2B (1 gene), 2C (4 genes), 2D (1 gene), 2E (1 gene),

and 3A (2 genes).

Table 1.9. Cytochrome P450 enzyme inhibitors and substrates of target drugs[1,2].

Compound	1A2		2C19		2D6		3A4	
	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate
amphetamine						X		
chlorpromazine					X	X		
citalopram								X
clozapine		X			X			
codeine						X (morphine)		X
flunitrazepam								X
fluvoxamine	X		X				XX	
fluoxetine			X		X	X	X	
haloperidol					X			
olanzapine		X						
oxazepam								
nefazodone							X	X
norfluoxetine						X	XX	
paroxetine					X	X	X	
risperidone						X		
sertraline					X		X	X
thioridazine						X		
venlafaxine						X		

[1] (Beno, 1996; Wilkins, 1996). [2] Potency of inhibition: X = weak inhibitor; XX = potent inhibitor.
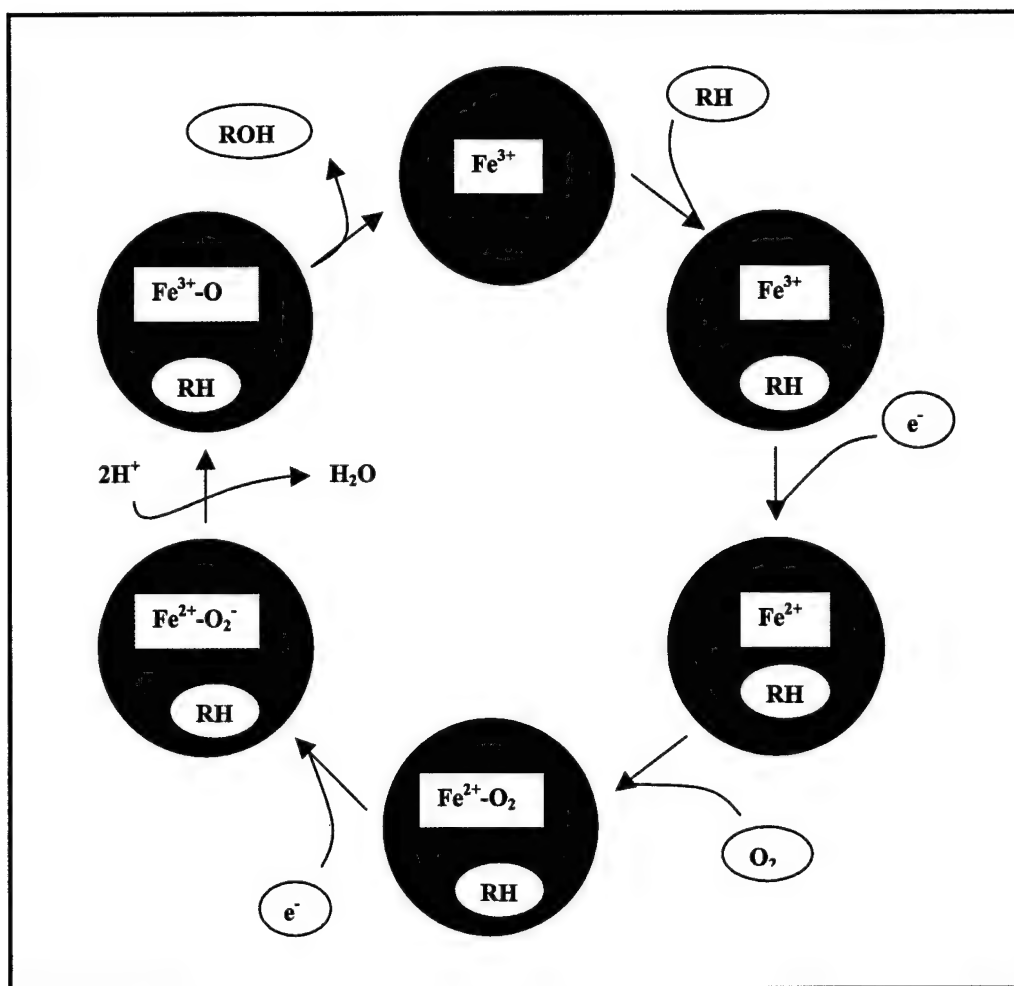
Figure 1.15. Cyclic monooxygenation reaction involved in CYP450 enzyme metabolism (after Moody) (Moody, 1996). On atom of oxygen is incorporated into the substrate, resulting in reduction of iron, binding of O_2, reduction of O_2, consequent substrate oxygenation, and release of product.

Autoinhibition, discussed in section 1.3.2, occurs when a drug is both a substrate for and inhibitor of a particular enzyme. Many of the antipsychotics are substrates or inhibitors of CYP2D6, while the antidepressants included in this work are substrates and/or inhibitors of 2C19, 3A4, and 2D6 (predominantly by 3A4 and 2D6) (Beno, 1996; Rollins, 1996; Wilkins, 1996).

The same enzymes that mediate metabolism of psychiatric drugs metabolise many of the drugs of abuse mentioned in section 1.3.4. There are some differences among the classes, however. The CYP450 2D subfamily catalyses ring hydroxylation of amphetamines (Law et al, 2000), while the 3A4 subtype has been shown to be the major enzyme responsible for hydroxylation and demethylation of flunitrazepam and most other benzodiazepines undergoing demethylation and hydroxylation in human liver microsomes (Hesse et al, 2001; Tanaka, 1999).

The 3-O-demethylated metabolites of codeine and dihydrocodeine, morphine and dihydromorphine, are formed by CYP2D6 (Eckhardt et al, 2000). Cocaine metabolism is more complicated. In a study of mouse and human liver microsomes, Pellinen et al suggested the CYP3A subfamily plays a prominent role in the N-demethylation of cocaine for a variety of reasons (Pellinen et al, 1994). However, a later study concluded that although both CYP2B and 3A catalyse the initial oxidation of cocaine in rats, only the 2B subfamily is involved in further oxidation that leads to potential toxicity (Poet et al, 1996). Finally, Powers and Shuster demonstrated that CYP 2A5 is the major isoform responsible for norcocaine N-hydroxylation, and that cocaine taken chronically can induce its own metabolism as opposed to the autoinhibition exhibited by the SSRIs (Powers and Shuster, 1999).

So-called "poor metabolisers", that is, people in whom a particular CYP450 enzyme is not expressed, run the risk of toxic adverse reactions in the presence of multiple substrates for that enzyme due to competitive inhibition. Since many patients on SSRIs are also co-prescribed benzodiazepines to mitigate the stimulant effects of the antidepressant, potential exists for metabolic inhibition to occur. This is a danger with substrates or inhibitors of CYP2D6, as

genetic polymorphism has been observed with this enzyme (Kroemer and Eichelbaum, 1995). Adverse reactions can occur when existing enzymes are saturated by the presence of multiple substrates, and are more likely in poor-metabolisers because if other competing substrates are present, enzyme saturation occurs at lower drug concentrations. Similarly, competitive inhibition is less of a concern in "normal" metabolisers.

Although isoenzyme inhibition can occur whenever two or more substrates or inhibitors of a particular enzyme are present, only some are clinically relevant. Most of these involve CYP2D6. For example, this enzyme is responsible for the metabolism for many neuroleptics. Because of this, a patient taking a neuroleptic known to be metabolised by 2D6 (such as chlorpromazine or thioridazine) who also takes an antidepressant such as paroxetine, fluoxetine, or sertraline which inhibits this enzyme, may have increased plasma neuroleptic concentrations which would increase the risk of extrapyramidal symptoms (Ciraulo and Shader, 1990; Goldberg, 1996). Another example involves CYP1A2. Bleeding disorders arising from changes in platelet serotonin concentrations may be worsened in a subject taking fluvoxamine, an inhibitor of 1A2, and warfarin, a substrate for this enzyme. When these drugs are co-prescribed, both warfarin concentrations and prothrombin times can be increased. Bradycardia has been reported to worsen due to impaired metabolism of β-adrenergic blockers in the presence of fluoxetine (Drake and Gordon, 1991; Hayes et al, 1989; Walley, 1993). The subject of clinically relevant isoenzyme metabolism of the target psychiatric drugs is explored in more detail in Chapter 8.

Drugs such as fluvoxamine or codeine, which are metabolised by more than one enzyme, are less likely to be affected by metabolic inhibition stemming from saturation of one enzyme. For

example, drugs metabolised by more than one enzyme, such as codeine, are less affected by saturation of a particular enzyme (Rudy, 1998).

1.5. Physiological, adverse effects and toxicology of antipsychotics and antidepressants in postmortem cases

1.5.1. Antidepressants

The main physiological effect of serotonin-active antidepressants is to elevate mood. The manner in which they do this, however, is variable since each type targets different receptor subtypes and/or neurotransmitter transporters. Generally speaking, the antidepressants affect serotonin at the presynaptic neuron. SSRIs block the serotonin transporter (5-HTT), while monoamine oxidase inhibitors antagonize monoamine oxidase-A or -B (MAOA or MAOB). Dual-acting antidepressants such as venlafaxine achieve their effect by also interacting with α_2-adrenoceptors at the nerve terminal (Veenstra-VanderWeele et al, 2000).

After repeated treatment with these drugs, complex changes occur in serotonin receptors, including an inconsistent loss of forebrain 5-HT_1 or 5-HT_2 receptors with ill-defined consequences (Heninger and Charney, 1987; Wamsley et al, 1987). Presynaptic 5-HT_{1A} or 5-HT_{1D} autoreceptors become desensitised after repeated exposure to paroxetine (Chaput et al, 1991). In contrast to the classical TCAs, serotonergic antidepressants have little effect on the autonomic nervous system. These drugs, like the TCAs, can cause orthostatic hypotension or

induce arrhythmias, due to vasoconstriction or vasodilation potentiated by increased serotonin concentrations. Venlafaxine treatment may also lead to slight elevations in blood pressure due to serotonin-induced vasoconstriction (Thase, 1998).

Some differences in time to onset of effect exist between the SSRIs. A meta-analysis of 20 short-term comparative studies of SSRIs showed no difference in efficacy between drugs, although a slower onset of action for fluoxetine was observed at weeks 2 and 3 (Edwards and Anderson, 1999). The authors suggested fluoxetine's therapeutic effect had not caught up with that of the other SSRIs by week 6. They recommended, therefore, that it be avoided in cases where a rapid onset of action is desired or in agitated patients. However, fluoxetine was deemed to be the drug of choice for patients who are poorly compliant or who are prone to withdrawal symptoms due to its (and norfluoxetine's) long terminal half-lives. They also recommended avoiding citalopram in suicidal patients due to its association with a comparatively large number of deaths (30%) caused by intentional overdoses (Edwards and Anderson, 1999).

While appearing to be much safer in overdose than their tricyclic predecessors, serotonergic antidepressants have been found to have significant side effects, since serotonin plays an important role throughout the body. However, deaths solely due to an SSRI are extremely rare. A common side effect associated with SSRIs is Syndrome of Inappropriate Secretion of Antidiuretic Hormone (SIADH), manifested by impaired water excretion and associated hyponatremia and hypoosmolality, resulting in lethargy, anorexia, nausea, vomiting, muscle cramps, coma, convulsions and death (Beno, 1996; Liu et al, 1996).

SIADH and hyponatremia are both observed in subjects taking fluoxetine, fluvoxamine,

sertraline, and paroxetine (Goeringer et al, 2000a). Sexual dysfunction is another common side

effect of all antidepressants studied, although the degree varies greatly between the different

drugs. Drugs associated with the highest incidence of sexual dysfunction include venlafaxine,

citalopram, and fluoxetine, while the fewest were reported with nefazodone (Montejo et al,

2001).

Both nefazodone and venlafaxine are known to have multiple sites of antidepressant action, but

they lack activity at the histaminic and acetylcholinergic receptor sites associated with the

unpleasant side effects or difficulty in tolerating long-term medication commonly seen in people

taking TCAs (Horst and Preskorn, 1998). With venlafaxine, the most common side effects early

in treatment are insomnia, nausea, agitation and dry mouth. At high doses, some elderly patients

may experience hypertension at doses above 150 mg/day (Staab and Evans, 2000). However,

both nefazodone and venlafaxine present important alternatives to the SSRIs in the treatment of

depression.

One peculiarity reported in conjunction with prolonged use of fluoxetine is the so-called

"stimulant syndrome" (Breggin, 1994). This term describes a phenomenon in which some

patients undergoing fluoxetine therapy experience feelings of intense agitation and sometimes

rage, often culminating in a violent act. The violence is sometimes directed towards themselves,

as in the case of suicide. However, sometimes the violence is directed outwards in the form of

homicide. Hundreds of cases have been reported, including a man who thought his cow was

leaning to one side and tried to straighten out the animal by backing the tractor into it. Another

report involved a woman who became enraged when her husband didn't appreciate her sitting on the roof for hours cleaning it by hand because she thought it was dirty (Breggin, 1994). In another case, the father of two children, known to be "a kind and gentle man who had never been suicidal or aggressive", taking fluoxetine for stress and fatigue, stabbed his wife to death without warning or provocation. After inflicting multiple wounds on her, he killed himself (Breggin, 1994).

A review of the scientific literature on the subject of SSRI-induced aggressive behaviour, however, provides perhaps a more objective view. Although a small proportion of patients treated with SSRIs may become akathisic or increasingly anxious during the initial phase of treatment, no increased susceptibility to aggression or suicidality can be connected with fluoxetine or any other SSRI (Walsh and Dinan, 2001). In fact, treatment with SSRIs or other serotonergic drugs has successfully reduced aggression in some cases, since these drugs have a positive effect on the serotonergic dysfunction implicated in aggressive behaviour (Buck, 1995; Chengappa et al, 2000; Fuller, 1997; Walsh and Dinan, 2001). The authors of one study hypothesized that a higher number of violent suicides in SSRI-treated patients may be the result of SSRIs' inherent lower lethality compared to traditional antidepressants, necessitating the use of additional means to complete the act of suicide (Frankenfield et al, 1994). Another possibility is that after noting that a particular patient is exhibiting a high degree of impulsiveness or aggression, physicians switch him or her to SSRIs, creating a selection bias for more violence-prone individuals. The problem of aggression in SSRI-treated patients is dangerous in the early stages of treatment since it takes several weeks for maximum antidepressant effect to occur with these drugs. Patient observation is thus most important during these early weeks of treatment.

The major mechanism of drug toxicity associated with SSRIs is through serotonergic excess resulting from a variety of causes, which may manifest as serotonin syndrome (SS), stemming from brainstem and spinal cord activation of the 5-HT_{1A} receptor. The occurrence of SS has been documented in cases involving moclobemide (or another MAOI) in combination with one or more SSRIs, pure SSRI overdoses, combinations of SSRIs at therapeutic doses, and with serotonin-active TCAs, either alone or in combination with other SSRIs (Brubacher et al, 1996; Chan et al, 1998; Corkeron, 1995; Diamond et al, 1998; Drummer, 1998; Gillman, 1998; Hodgman et al, 1997; Kolecki, 1997a, 1997b; McIntyre et al, 1997; Mekler and Woggon, 1997; Molaie, 1997; Singer and Jones, 1997; Weiner et al, 1998; Weiss, 1995). However, postmortem diagnosis of this condition is difficult unless documented clinically prior to death (Goeringer et al, 2000a, 2000b). For this reason, it may not appear on most death certificates even when this syndrome is directly implicated. SS bears some resemblance to neuroleptic malignant syndrome, the differential diagnosis of which will be discussed in Section 1.5.3.

Although acute intoxication in healthy individuals is uncommon, the coronary vasoconstriction or dilation associated with elevated serum serotonin levels can also be important in subjects with a pre-existing cardiac pathology such as atherosclerotic cardiovascular disease (ASCVD). Subjects with chronic heart conditions comprise a significant percentage of postmortem cases involving serotonergic drugs, although the existence of natural disease is often not linked to cause of death (Fu et al, 2000; Goeringer et al, 2000a, 2000b; Levine et al, 1994; Parsons et al, 1996; Vermeulen, 1998). The significance of this is uncertain, but it appears that the existence of heart disease may cause individuals to be more susceptible to death as a result of adverse drug reactions which might otherwise not be fatal.

The relative safety of serotonergic antidepressants is evidenced by the fact that few deaths in which an SSRI was detected alone have been reported (Anastos et al, in press; Levine et al, 1994; Logan et al, 1994; Worm et al, 1998). The deaths in such cases were clearly attributable either to violent means or natural disease, and therefore the presence of the SSRI was more of an anecdotal finding. Two of these cases involved sertraline, with blood concentrations ranging 0.36-0.83 mg/L for sertraline and 0.08-1.4 mg/L for N-desmethylsertraline (NDS) (Levine et al, 1994; Logan et al, 1994). Citalopram was detected in the other two reports at concentrations ranging 0.8-6.2 (Anastos et al, in press; Worm et al, 1998). Two reports of suicide caused by venlafaxine-only overdose have been reported. In these cases, peripheral blood venlafaxine concentrations were 44 and 65 mg/L respectively, with O-desmethylvenlafaxine (ODV) concentrations of 50 and 7.1 mg/L (Levine et al, 1996; Parsons et al, 1996).

When taken in combination with other drugs with serotonergic activity or which are substrates and/or inhibitors for the relevant CYP450 isoenzyme, toxicity is more likely to occur. Numerous fatalities have occurred in subjects who ingested a serotonergic antidepressant in combination with another drug of the type mentioned earlier. A review has been published of postmortem blood concentrations and toxicology in a number of death investigation cases in which SSRIs were detected (Goeringer et al, 2000a). A number of studies of the forensic toxicology of individual serotonergic drugs have also been published (Anastos et al, in press; Bidanset et al, 1999; Fu et al, 2000; Jaffe, 1997; Kunsman et al, 1999; Levine et al, 1994; Levine et al, 1996; Logan et al, 1994; McIntyre et al, 1997; Parsons et al, 1996; Singer and Jones, 1997; Vermeulen, 1998; Win, 1994; Worm et al, 1998). The means and ranges of reported concentrations for each antidepressant detected in these cases are summarised in Table 1.10.

Table 1.10. Tissue concentrations of antidepressants[1,2].

References	Blood[3]	Brain[4]	Bile	Vitreous	Urine	Liver	Gastric (mg)
Citalopram							
(Anastos et al, in press) (n=14)[5]	0.49 (0.1-1.3)	--	2.7 (0.8-6.0)	0.19 (0.1-0.4)	--	3.5 (1.3-18)	--
(Fu et al, 2000) (n=1)[4]	0.88 F, 1.2 H	5.9	--	--	--	8.1	36
(Worm et al, 1998) (citalopram alone, n=4)[4]	3.9 (2.0-6.2)	--	--	--	--	40 (24-55)	--
(Worm et al, 1998) (citalopram + other, n=20)[4]	1.2 (0.6-5.2)	--	--	--	--	19 (1.9-36)	--
Fluoxetine							
(Jaffe, 1997) (n=16)[5]	0.78 (0.13-3.0)	--	4.8 (0.58-15)	0.1 (0.0-0.24)	--	27 (1.0-160)	--
(Goeringer et al, 2000) (n=60)[5]	0.83 (<0.05-6.7)	--	--	--	--	--	0.14
(Singer and Jones, 1997) (n=1)[4]	0.41	--	--	--	--	8.2	3.0
Fluvoxamine							
(Bidanset et al, 1999) (n=1)[4]	2.8	42	--	--	24	110	--
(Goeringer et al, 2000) (n=5)[5]	1.9 (0.19-5.8)	--	--	--	--	--	--
(Kunsman et al, 1999) (n=4)[4]	2.0 F / 1.5 H	--	--	0.78 (0.16-1.9)	7.0	--	28
(Singer and Jones, 1997) (n=1)[5]	0.46	--	--	--	--	24	2.4
Nefazodone—no published data available							

References	Blood[3]	Brain[4]	Bile	Vitreous	Urine	Liver	Gastric (mg)
Paroxetine							
(Jaffe, 1997) (n=7)[5]	0.2	--	8.0	negative	--	7.0	--
(Goeringer et al, 2000) (n=28)[5]	0.34 (<0.05-3.8)	--	--	--	--	--	--
(Singer and Jones, 1997) (n=2)[4]	1.4 (1.1-1.6)	--	--	--	--	10 (5.1-15)	2.1 (0.7-3.4)
(Vermeulen, 1998) (n=3)[4]	2.9 F 3.0 (1.4-4.0) H	--	--	--	5.5 (<0.5-10.5)	80 (16-110)	100
(Win, 1994) (n=1)[4]	0.24	--	--	--	--	3.5	--
Sertraline							
(Jaffe, 1997) (n=15)[5]	0.38 (0.07-2.0)	--	9.4 (1.3-29)	0.16	--	24 (0.45-98)	--
(Goeringer et al, 2000) (n=75)[5]	0.41 (0.21-4.2)	--	--	--	--	--	--
(Levine et al, 1994) (sertraline alone, n=3)[5]	0.35 F 0.34 H (0.32-0.36) H	--	2.5 (2.1-2.8)	negative	0.07	11 (3.9-19)	--
(Levine et al, 1994) (sertraline + other, n=4)[5]	0.43 F (0.23-0.82) F 0.35 H (0.23-0.46) H	--	6.0 (2.1-10)	0.03	0.33 (0.03-0.63)	14 (4.7-20)	
(Logan et al, 1994) (n=3)[5]	0.63 F 0.66 (0.44-0.84)	--	--	--	trace	--	--

References	Blood[3]	Brain[4]	Bile	Vitreous	Urine	Liver	Gastric (mg)
Sertraline (continued)							
(McIntyre et al, 1997) (n=1)[4]	0.9	--	negative	0.5	--	3.2	--
(Singer and Jones, 1997) (n=2)[4]	1.5	--	--	--	--	10 (1.8-19)	4.1 (0.43-7.8)
Venlafaxine							
(Jaffe, 1997) (n=11)[5]	0.99 (0.13-2.1)	--	5.3 (0.4-20)	1.6 (0.13-4.5)	--	14 (0.47-55)	--
(Levine et al, 1996) (venlafaxine alone, n=1)[4]	44	--	--	--	--	--	--
(Levine et al, 1996) (venlafaxine + other, n=2)[4]	46 F 45 H (6.6-84) H	--	200 (100-290)	--	400 (150-640)	230 (34-430)	--
(Parsons et al, 1996) (venlafaxine alone, n=1)[4]	65 F 85 H	--	--	23	20	220	--
(Parsons et al, 1996) (venlafaxine + other, n=1)[4]	17 F 30 H	--	--	11	73	430	--
(Singer and Jones, 1997) (n=2)[4]	9.1 F, 1.1 H	--	--	0.87	--	8.8	320

[1] (Anastos et al, in press; Bidanset et al, 1999; Fu et al, 2000; Goeringer et al, 2000; Jaffe, 1997; Kunsman et al, 1999; Levine et al, 1994; Levine et al, 1996; Logan et al, 1994; McIntyre et al, 1997; Parsons et al, 1996; Singer and Jones, 1997; Vermeulen, 1998; Win, 1994; Worm et al, 1998). [2] Mean concentrations (ranges shown in parentheses); concentrations in mg/L or mg/kg. [3] Blood concentrations are from femoral sites unless otherwise stated; F = femoral, H = heart. [4] Cases where target drugs played a major role. [5] Reports of a variety of types of death where target drugs were detected. [4] Sampled region not specified.

Eight of these studies detailed cases in which the target psychiatric drugs played a major role in causing death. The remaining studies describe a variety of types of death in which the target psychiatric drugs were detected.

The study by Worm et al compared a number of deaths in which citalopram toxicity alone caused death to others in which toxicity due to citalopram in combination with other drugs had occurred (Worm et al, 1998). Deaths reported by Levine and Parsons also included antidepressant-only cases as well as cases in which other drugs were detected (Levine et al, 1996; Parsons et al, 1996). In the fluvoxamine-related death reported by Bidanset et al, imipramine and diazepam were detected in blood at concentrations of 0.12 and <0.1 mg/L, respectively (Bidanset et al, 1999). In the death described by Win, amitriptyline was also detected in blood at a concentration of 0.43 mg/L (Win, 1994). In all other deaths, multiple other drugs were also reported.

The tissue:blood concentration ratios for each psychiatric drug in these studies is shown in Figure 1.16. The highest drug concentrations were detected in liver and bile. In brain specimens analysed in two studies (Bidanset et al, 1999; Fu et al, 2000), drug concentrations were also quite high relative to other tissues. However, the brain region sampled was not specified in either study. The lowest concentrations were detected in vitreous fluid.
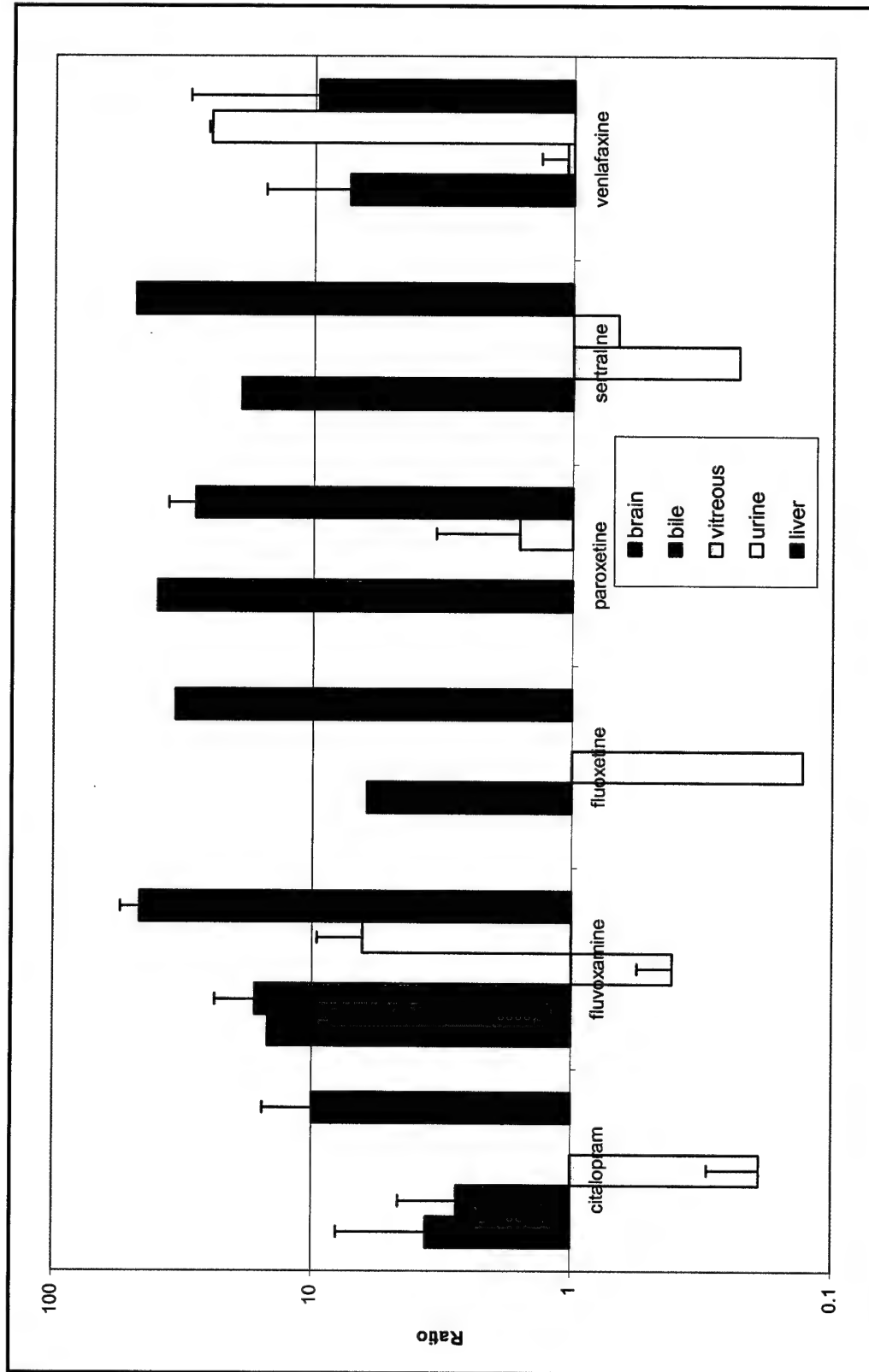
Figure 1.16. Literature concentration ratios of target psychiatric drugs in tissues compared with blood (means ±SD's).

1.5.2. Antipsychotic drugs

Antipsychotic drugs as a class share many pharmacological effects. They exert sedation (particularly noticeable early in treatment), and they possess some antianxiety effect. The behavioural and motor effects of these drugs were summarised by Fielding and Lal (Fielding and Lal, 1978) and Janssen and Van Bever (Jannsen and Van Bever, 1978). These effects include diminished exploratory behaviour, inhibited conditioned avoidance behaviours, and loss of appetite. Antipsychotics have inconsistent effects on sleep, but tend to normalise the sleep disturbances which characterise many psychoses.

Many antipsychotics, particularly phenothiazines belonging to group 1 (see Section 1.3.3), can lower the seizure threshold and induce discharge patterns in the EEG that are associated with epileptic seizure disorder (Itil, 1978). The butyrophenones (such as haloperidol) have variable effects that cause seizures, but clozapine has a clearly dose-related risk of seizure induction in nonepileptic patients (Hardman and Limbird, 1996).

In addition to these effects, endocrine changes as a result of neuroleptic effect on the hypothalamus or pituitary are common; particularly, an increase in prolactin secretion in humans is frequently observed (Ben-Jonathan, 1985). There is less likelihood of acute extrapyramidal side effects with clozapine, thioridazine, or low doses of risperidone. There are six varieties of neurological syndromes characteristic of antipsychotic drugs, all of which have been reviewed extensively (Baldessarini et al, 1980; Baldessarini, 1984; Kane et al, 1992; Tarsy and Baldessarini, 1986). Four of these (acute dystonia, akathisia, parkinsonism, and the rare

neuroleptic malignant syndrome, or NMS) usually appear soon after administration of the drug. The other two neurological side effects, rare perioral tremor and tardive dyskinesia or dystonia, are late appearing syndromes that occur after prolonged treatment. Other dangerous effects of antipsychotics are seizures, agranulocytosis, and pigmentary degeneration of the retina, all of which are rare.

NMS resembles a very severe form of parkinsonism with catatonia, fluctuations in the intensity of coarse tremor, signs of autonomic instability (labile pulse and blood pressure, hyperthermia), stupor, elevation of creatine kinase in plasma, and sometimes myoglobinaemia (Hardman and Limbird, 1996).

Other serious adverse reactions associated with antipsychotics arise as a consequence of interactions with other drugs. However, these reactions are usually not fatal. Chlorpromazine increases the miotic and sedative effects of morphine, and may also increase its analgesic actions. It also greatly increases the respiratory depression produced by pethidine, and may do so with other opioids (Goff and Baldessarini, 1993). Thioridazine may partially nullify the inotropic effect of digitalis, which can cause myocardial depression, decreased efficiency of repolarization, and increased risk of tachyarrhythmias. The antimuscarinic action of clozapine and thioridazine can cause tachycardia and enhance the peripheral and central effects (e.g. confusion, delirium) of other anticholinergic agents, such as the tricyclic antidepressants and antiparkinsonian agents (Segal et al, 1979).

Numerous fatalities involving neuroleptic drugs have been reported in the literature (Allender, 1985; Baselt, 1976; Baselt et al, 1978; Baselt, 1981; Bonnichsen et al, 1970; Gerber and Cawthon, 2000; Levine et al, 1991; Meeker et al, 1992; Quai et al, 1985; Springfield and Bodiford, 1996; Street, 1981). In fatalities involving chlorpromazine, blood concentrations may be similar in cases involving chronic high-dose therapy and those in fatal overdoses. Differentiation between the two case types can be made by determining liver concentrations, which generally do not exceed 20 mg/kg when chlorpromazine is being used therapeutically (Baselt and Cravey, 2000). The same may be said of the other antipsychotics under investigation. Table 1.11 summarises published postmortem tissue distribution data from fatalities involving antipsychotic drugs. The deaths in all of these studies, except the thioridazine report published by Baselt et al (Baselt et al, 1978), were ascribed at least partially to antipsychotic drug toxicity. In Baselt's unpublished results from 1981, chlorprothixene was also detected in the blood at a concentration of 1.4 mg/L (Baselt, 1981). In the risperidone overdose reported by Springfield and Bodiford, buspirone was also detected in the blood (0.05 mg/L) and urine (0.1 mg/L), and perphenazine was detected in the urine (2.0 mg/L) (Springfield and Bodiford, 1996). In the remaining studies, the antipsychotic drug cited was the only drug detected.

Figure 1.17 shows the tissue:blood concentration ratios of the target antipsychotic drugs. Like studies of the tissue distribution of antidepressant drugs, the highest drug concentrations were detected in liver for all antipsychotics under investigation. Unfortunately, brain specimens were not analysed for all these drugs, and the regions sampled were not specified in two of these reports. Although considerably lower than those detected in liver, the brain concentrations in both studies were considerably higher than in blood.
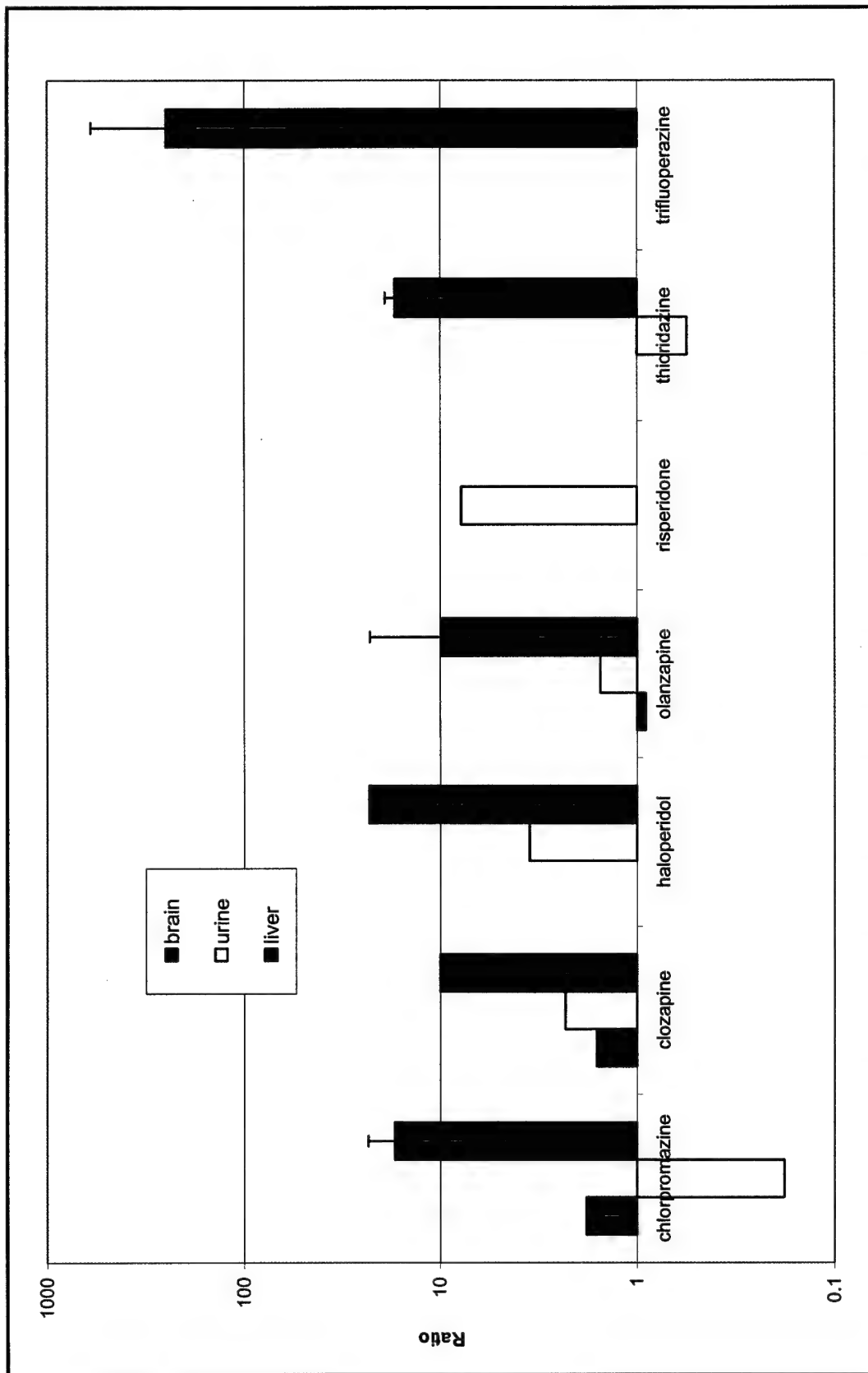
Figure 1.17. Literature concentration ratios of target antipsychotics drugs in tissues compared with blood (means ±SD's).

Table 1.11. Tissue concentrations of antipsychotic drugs in fatalities[1,2,3].

Reference	Blood[4]	Brain	Liver	Urine	Gastric
Chlorpromazine					
(Baselt, 1976) (n=1)[5]	6.6	12	84	1.2	21
(Bonnichsen et al, 1970) (n=8)[3]	17 (3-35)	--	366 (54-2110)	--	--
Clozapine					
(Meeker et al, 1992) (n=3)[5]	4.8 (1.6-7.1)	7.5	48 (19-82)	11	20 (1.1-54)
Flupenthixol decanoate: no published data available					
Fluphenazine					
(Baselt, 1981) (n=1)[3]	not detected	--	23	--	not detected
(Baselt, 1976) (n=1)[5]	not detected	--	5	--	1.2
Haloperidol					
(Levine et al, 1991) (n=1)[5]	1.9	--	44	6.6	67
Olanzapine					
(Gerber and Cawthon, 2000) (n=2)[5]	0.46 (0.24-0.68)	not detected	not detected	--	8.6 (0.20-17)
(Merrick et al, 2001) (n=1)[5]	0.40 H	0.36	0.61	0.35	0.33
Risperidone					
(Springfield and Bodiford, 1996) (n=1)[3]	1.8	--	--	14	1.0
Thioridazine					
(Baselt et al, 1978) (n=14)[4]	3.5 (0.6-13)	--	65 (1.0-513)	--	--
(Allender, 1985) (n=8)[5]	2.5 (0.3-8.5)	--	39 (3.6-150)	1.4 (0.7-2.0)	390 (0.3-2500)

Reference	Blood[3]	Brain	Liver	Urine	Gastric
		trifluoperazine			
(Street, 1981) (n=1)[5]	0.06	--	0.31	--	--
(Quai et al, 1985) (n=1)[5]	0.40	--	200	--	--

[1] (Allender, 1985; Baselt, 1976; Baselt et al, 1978; Baselt, 1981; Bonnichsen et al, 1970; Gerber and Cawthon, 2000; Levine et al, 1991; Meeker et al, 1992; Quai et al, 1985; Springfield and Bodiford, 1996; Street, 1981)

[2] Mean concentrations with ranges shown in parentheses; all concentrations in mg/L or mg/kg. [3] Combined drug toxicities ascribed partially due to the antipsychotic. [4] Deaths ascribed to causes other than drug overdose.

[5] Antipsychotic was only drug detected. [6] Blood concentrations are from femoral sites unless otherwise stated.

1.5.3. Differential diagnosis of serotonin syndrome versus neuroleptic malignant syndrome

Serotonin syndrome (SS) and neuroleptic malignant syndrome (NMS) are two potentially fatal complications of psychiatric drug therapy. As its name suggests, SS occurs in the setting of serotonergic drug therapy (predominantly antidepressants, although other serotonin-active drugs may be implicated). NMS, on the other hand, occurs in patients being treated with dopaminergic drugs (typically neuroleptics). NMS can persist for more than a week after stopping the offending agent, and the associated mortality rate is relatively high (10%) (Levenson, 1985). Consequently, immediate medical attention is required. Unfortunately, NMS bears strong resemblance to SS, so it is important that physicians are able to differentially diagnose the two syndromes. A comparison of symptoms of each syndrome is shown in Table 1.12. In NMS, spontaneous movements and complex behaviours are suppressed while spinal reflexes and unconditioned nociceptive-avoidance behaviours are left intact (Malone, 1992).

SS may be differentially diagnosed by noting the absence of neuroleptics and presentation of muteness, rigidity, and autonomic dysfunction, none of which is indicative of NMS. Kline et al theorized that SS and NMS may be two forms of a more generalised hyperthermic syndrome (Kline et al, 1989). Fatalities in which antidepressants are detected either alone or in combination with neuroleptics, however, makes the possibility of toxicity due to either syndrome an important consideration.

Table 1.12. Common symptoms of neuroleptic malignant syndrome (NMS) and serotonin syndrome (SS)[1].

Symptom	NMS	SS
mental status changes	X	X
restlessness	X	X
myoclonus		X
hyperreflexia		X
diaphoresis	X	X
shivering	X	X
hyperthermia[2]	X	X
tremor	X	X
diarrhoea		X
incoordination	X	X
autonomic dysfunction	X	
rigidity	X	
muteness	X	

[1] (Kline et al, 1989). [2] Not necessarily present in SS, or may be delayed in onset.

1.5.4. Drugs of abuse

Many drugs of abuse, including the designer amphetamine (+)-3,4-methylenedioxyamphetamine (MDMA, "ecstasy"), hallucinogenics (like lysergic acid diethylamide (LSD) and psylocibin), and the anxiolytic benzodiazepines affect serotonin dynamics (Sibille et al, 2000). Evidence points to the possibility that dopamine and noradrenaline are also affected by drugs of abuse (particularly by amphetamine, MDMA, and cocaine) (Al-Sahli et al, 2001; Mayerhofer et al, 2001; Nath et al, 2001). The effects of selected drugs of abuse on monoamine systems has been presented in section 1.3.4. This section reviews the physiological effects of drugs of abuse, as well as their associated side effects and toxicity.

1.5.4.1. Amphetamine-like compounds

Amphetamines increase synaptic dopamine and noradrenaline primarily by stimulating presynaptic release of these monoamines. One of the most prominent effects of amphetamine-like compounds is an increase in blood pressures and the causation of alterations in heart function by their sympathomimetic activity on α-adrenoceptors at higher doses or with regular use. Some users may experience myocarditis, vasospasm, oedema, necrosis, pain, and paraesthesia. The smooth muscle can be affected in the same way as the heart, leading to tremor, contraction of the bladder or sphincter and reduced gut motility, leading to delayed drug absorption (Drummer, 2001).

Centrally, amphetamines are known to cause an elevation of mood in addition to increased alertness, confidence, and mental and physical strength. However, the improved performance may be more perceived than real. The neurobiological basis of amphetamine-induced arousal is believed to involve β-noradrenoceptors in the forebrain (Berridge and Morris, 2000). Other effects are a reduction in appetite and upset of thermoregulatory settings in the brain stem. In one study involving repeated administration of MDMA, changes in serotonin, dopamine, and noradrenaline levels in rat brains were observed. In the whole forebrain, serotonin levels were reduced at both two and four weeks after cessation of treatment. Noradrenaline concentrations were also decreased in the nucleus accumbens, but dopamine concentrations increased in this region upon cessation of treatment (Mayerhofer et al, 2001). The authors hypothesised that decreased noradrenaline and/or serotonin concentrations following MDMA abuse may subsequently trigger dopamine augmentation in the nucleus accumbens. The reinforcing and addictive properties of amphetamines appear to be associated with dopamine in this region of the brain (Carboni et al, 2001).

The amphetamines in general, and particularly methamphetamine and MDMA, have been shown to be neurotoxic and cause long-lasting or permanent nerve damage to dopamine and serotonin-containing nerves in the brain. The mechanism by which this occurs is not fully understood, but Molliver and colleagues found that MDMA and related compounds cause release of serotonin as well as cause acute depletion of 5-HT from most axon terminals in the forebrain (Molliver et al, 1990). Specifically, they noted

1) increased calibre, swollen varicosities, fragmentation, and dilated proximal axon stumps exhibited in 5-HT axons at 36-48 hours post-treatment, and

2) fine 5-HT axon terminals were persistently lost after drug administration while beaded axons and raphe cell bodies were spared.

Two to eight months post-treatment, however, progressive serotonergic re-innervation of neocortex along a fronto-occipital gradient was observed.

CNS side effects of amphetamine use include restlessness, dizziness, tremor, hyperactive reflexes, talkativeness, tenseness, irritability, weakness, insomnia, fever, headache, chilliness, diaphoresis, pallor or flushing, and sometimes euphoria. Confusion, aggressiveness, changes in libido, anxiety, delirium, paranoid hallucinations, panic states, and suicidal or homicidal tendencies can occur, especially in mentally ill patients. Nath et al observed an acceleration of HIV dementia with the non-parenteral use of methamphetamine and cocaine (Nath et al, 2001). Fatigue and depression generally follow central stimulation. It has been hypothesised that the

sleep disorders and mood changes commonly reported in amphetamine users may stem from impairment in serotonin-related phase shifting of the circadian clock (Biello and Dafters, 2001).

The cardiovascular system is commonly affected with amphetamine use, symptoms of which include palpitation, cardiac arrhythmias, anginal pain, hypertension or hypotension, and circulatory collapse. Al-Sahli et al observed MDMA-related potentiation of the actions of noradrenaline, and suggested this finding may have implications for cardiac morbidity associated with MDMA use (Al-Sahli et al, 2001).

Gastrointestinal side effects include dry mouth, metallic taste, anorexia, nausea, vomiting, diarrhoea, and abdominal cramps. Croft et al showed that long-term use of MDMA causes impairment of serotonin-related attenuation of neural response to auditory stimuli in humans, and that such impairment does not pre-date use of the drug, as previously thought (Croft et al, 2001). Acute toxic side effects associated with amphetamine use sometimes occur after as little as 2 mg, but are rare with doses under 15 mg. Severe reactions have occurred with a 30 mg dose, but some people have survived doses of 400 to 500 mg.

Deaths from amphetamine use alone are rare. However, the fatal poisonings that do occur usually terminate in convulsions and coma, with cardiac arrthymias being the main pathological findings. Chronic amphetamine intoxication causes symptoms similar to those seen with acute overdose, although abnormal mental conditions are more common. A psychotic reaction with vivid hallucinations and paranoid delusions, often mistaken for schizophrenia, is the most common serious effect (Hardman and Limbird, 1996). Cases of acute psychotic episodes after

ecstasy use, sometimes after only one dose, have been reported in the literature (Vaiva et al, 2001). MDMA disturbs dopaminergic function, leading to a sudden central hyperdopaminergic state, which may be related to the appearance of an acute psychotic disorder (Bone et al, 2000; Milas, 2000; Vaiva et al, 2001). Death usually does not occur immediately but after a period of several hours, during which the subject experiences agitation, hyperthermia, convulsions, unconsciousness, and respiratory and/or cardiac failure. There may be some connection between this phenomenon and NMS. Postmortem findings often include organ congestion and haemorrhage (Baselt and Cravey, 2000).

In fatalities resulting from acute oral or intravenous amphetamine administration reported in the literature, the actual dose of amphetamine was often not determined (Cox and Williams, 1996; Lora-Tamayo et al, 1997; Meyer et al, 1997). However, in one case the subject was known to have orally ingested 1.5-2 g of 8 % purity amphetamine cut predominantly with caffeine (120-160 mg active drug) at a nightclub (Cox and Williams, 1996). Amphetamine has occasionally been detected in combination with antidepressants and antipsychotics (Goeringer et al, 2000a). In such cases, the blood amphetamine concentrations often overlapped those in cases of pure amphetamine overdose. The range and mean blood concentrations in fatalities in which amphetamine was detected in the presence of psychiatric drugs is shown in Table 1.13.

Table 1.13. Selected references relating tissue concentrations of drugs of abuse in fatalities where target psychiatric drugs were detected[1,2].

References	Blood[3]	Brain	Liver	Urine
Amphetamine				
(Goeringer et al, 2000) (n=2)	1.1 (0.1-2.1)	--	--	--
Cannabis				
(Anastos et al, in press) (n=4)	--	--	--	detected[4]
Cocaine				
(Fu et al, 2000) (n=1)	0.03 F, 0.02 H	0.1	0.05	--
(Goeringer et al, 2000) (n=5)	0.38 (<0.05-1.4)	--	--	--
Codeine				
(Anastos et al, in press) (n=2)	0.3	--	--	3
(Bidanset et al, 1999) (n=1)	--	--	--	0.16
(Goeringer et al, 2000) (n=7)	1.0 (<0.05-7.0)	--	--	--
Diazepam				
(Anastos et al, in press) (n=3)	0.11 (0.04-0.2)	--	--	--
(Bidanset et al, 1999) (n=1)	<0.1	--	--	--
(Goeringer et al, 2000) (n=14)	0.21 (<0.05-0.69)	--	--	--
(Singer and Jones, 1997) (n=1)	0.23	--	--	--
Flunitrazepam (no studies in which psychiatric drugs also detected)				

References	Blood[3]	Brain	Liver	Urine
Heroin (measured as morphine)				
(Anastos et al, in press) (n=1)	--	--	--	18
(Bidanset et al, 1999) (n=1)	--	--	--	5.1
(Goeringer et al, 2000) (n=9)	0.16 (0.05-0.51)	--	--	--
Methadone				
(Goeringer et al, 2000) (n=15)	0.45 (0.14—1.2)	--	--	--
Methamphetamine				
(Anastos et al, in press) (n=1)	trace	--	--	--
(Goeringer et al, 2000) (n=2)	0.53 (0.21-0.85)	--	--	--
MDMA (no studies in which psychiatric drugs also detected)				
Morphine				
(Anastos et al, in press) (n=2)	0.12 (0.03-0.2)	--	--	--
(Goeringer et al, 2000) (n=11)	0.13 (<0.01-0.51)	--	--	--
Oxazepam (no studies in which psychiatric drugs also detected)				
Temazepam				
(Anastos et al, in press) (n=2)	0.8 (0.5-1.1)	--	--	--
(Goeringer et al, 2000) (n=1)	7.8	--	--	--

[1] (Anastos et al, in press; Bidanset et al, 1999; Fu et al, 2000; Goeringer et al, 2000; Singer and Jones, 1997). [2] Mean concentrations with ranges shown in parentheses; all concentrations in mg/L or mg/kg. [3] Blood concentrations are from femoral sites unless otherwise stated. [4] Cannabis was detected via EMIT.

Methamphetamine use has been detected in fatal poisonings following intravenous and oral administration. Blood concentrations in four deceased subjects who injected methamphetamine intravenously were as high as 0.8 mg/L (Cravey and Reed, 1970). Methamphetamine concentrations of 4.3 and 5.6 mg/L in blood and 28 and 320 mg/L in urine were measured in two deaths by oral ingestion of methamphetamine (Kojima et al, 1984; Patterson and Peat, 1976). In a child who developed hyperpyrexia and died 5.5 hours after taking a large amount of methamphetamine, concentrations of 40 mg/L in blood and 206 mg/kg in liver were detected (Cravey and Baselt, 1968). Methamphetamine (2.0 mg/L) and amphetamine (0.3 mg/L) were detected in a woman who died after nasal insufflation of methamphetamine. Like amphetamine, methamphetamine has occasionally been detected in the presence of antidepressants or antipsychotics (Anastos et al, in press; Goeringer et al, 2000a). In such cases, methamphetamine concentrations were on average lower than those in cases of methamphetamine-only toxicity. The blood methamphetamine concentrations detected in studies in which psychiatric drugs were also detected are summarised in Table 1.13.

Several deaths associated with MDMA use have been reported in the literature. In one case, a healthy 18-year-old woman who collapsed and died in ventricular fibrillation after ingesting approximately 150 mg of MDMA was found to have 1.0 mg/L of the drug in her blood, in combination with alcohol (0.04 g/100 mL) (Brown et al, 1986). Five other adult subjects who ingested large quantities of MDMA had postmortem blood concentrations of 0.6-2.8 mg/L (Forrest et al, 1994; Reynolds and Weingarten, 1983; Rohrig and Prouty, 1992; Suarez and Riemersma, 1988). A single report combined MDMA-psychiatric drug toxicity has been published. The case involved a 50 year-old male who developed marked hypertension,

diaphoresis, altered mental status, and hypertonicity after co-ingesting MDMA and the MAOI phenelzine (Smilkstein et al, 1987). However, at the time of writing, no published studies in which MDMA had been detected in combination with the target psychiatric drugs.

1.5.4.2. Cocaine

Cocaine is typically used for its stimulant effects, although it is used clinically and in emergency medicine as a local anaesthetic. Its use in anaesthesia has arisen due to its ability to block nerve impulses through decreasing or preventing the large transient increase in the permeability of excitable membranes to Na^+ that normally is produced by a slight depolarisation of the membrane (Strichartz and Ritchie, 1987). At high doses, this effect can lead to fatal cardiac arrhythmias (Drummer, 2001). Some users can continue intermittent use for years, while others become compulsive despite elaborate methods to maintain control. The reinforcing effects of cocaine and cocaine analogs correlate best with their effectiveness in blocking the dopamine transporter. Such blockade leads to increased dopaminergic stimulation at binding sites associated with inhibition of dopamine uptake on the nerve terminals in the striatum (Ritz et al, 1987). Hurd et al found acute cocaine doses increased dopamine overflow in drug-naïve animals (Hurd et al, 1990). After repeated dosing, however, the drug-induced elevation of DA was attenuated in animals that had previously been exposed. Hurd and colleagues theorised that the decreased DA may have been due to increased DA transporter activity and/or supersensitive receptors modulating DA release.

Cocaine also blocks noradrenaline and serotonin reuptake. A study of cocaine binding sites in caudate-putamen proposes the drug binds to the same receptor sites in this region of the brain as the SSRIs (Madras et al, 1989). This may mean that cocaine abusers taking SSRIs are in danger of side effects stemming from heightened serotonin concentrations. However, Walsh et al suggested fluoxetine may be useful in treating cocaine addiction as it decreased cocaine's positive mood effects and no adverse physiologic interactions were observed in subjects taking both drugs (Walsh et al, 1994).

Cocaine is sometimes taken in combination with heroin. This combination of drugs is commonly known as a "speedball" (Drummer, 2001). Users report an improved euphoria because of the combination. Additionally, cocaine reduces the signs of opioid withdrawal (Kosten, 1990) and heroin may reduce the irritability seen in chronic cocaine users. Alcohol is often also taken in combination with cocaine to reduce the irritability experienced during heavy cocaine use. An important metabolic interaction occurs in concomitant cocaine-alcohol use where some cocaine is transesterified to cocaethylene, which is as potent as cocaine in blocking dopamine uptake (Hardman and Limbird, 1996). Cocaine has been reported to produce a prolonged and intense orgasm if taken prior to intercourse, and its use is associated with often compulsive and promiscuous sexual activity. However, long-term cocaine use often results in reduced sexual drive, and complaints of sexual problems are common in cocaine users presenting for treatment.

In addition to the possibility of addiction, adverse drug reactions to cocaine include cardiac arrhythmias, myocardial ischaemia, myocarditis, aortic dissection, cerebral vasoconstriction, pulmonary dysfunction, and seizures. Cocaine-induced deaths are believed to be caused by the

direct cardiotoxic effect of the drug. Pregnant cocaine users may experience premature labour and abruptio placentae (Chasnoff et al, 1989). Reports of developmental abnormalities in infants born to cocaine-using women are confounded by prematurity, multiple drug exposure, and poor pre-and postnatal care. Psychiatric disorders, including anxiety, depression, and psychosis, are common in cocaine users, and although some of these undoubtedly existed before the cocaine use, many develop during the course of the drug abuse (McLellan et al, 1979).

A near-fatal case reported in the literature involved the unintentional rupture in the stomach of a 5 g packet of cocaine, resulting in unconsciousness and massive convulsions. The maximum blood concentration detected was 5.2 mg/L, but the patient survived with treatment (Suarez et al, 1977).

Cocaine concentrations observed in the tissues of victims who die due to acute toxicity vary greatly depending on the dosage, route of administration, period of survival and manner of storage of specimens. Intense paranoia, bizarre and violent behaviour, hyperthermia, and sudden collapse were observed in seven individuals whose postmortem blood concentrations averaged 0.6 mg/L (range, 0.1-0.9) (Wetli and Fishbain, 1985). Blood cocaine and BZE concentrations in 37 cocaine-related fatalities averaged 4.6 mg/L (range, 0.04-31) and 7.9 mg/L (range, 0.7-31), respectively (Spiehler and Reed, 1985). Postmortem blood concentrations averaged 3.0 mg/L in those who administered the drug intravenously, 4.4 mg/L in those who insufflated, and 9.2 mg/L in victims who took the drug orally (Wetli and Wright, 1979). Two victims of acute massive oral overdose were found to have blood cocaine concentrations of 52 and 211 mg/L (Amon et al, 1986; Winek et al, 1987).

The following mean cocaine concentrations were determined in 19 fatal cases that occurred after ingestion, inhalation or injection of from 160 mg (intravenously) to as much as 26 g (orally) of cocaine: 5.3 mg/L both in blood and brain, 4.2 mg/L in liver, 13 mg/L in kidney, and 42 mg/L in urine (Bednarczyk et al, 1980; DiMaio and Garriott, 1978; Gottshalk, 1977; Griffin, 1975; Lundberg et al, 1977; McCurdy and Jones, 1973; Poklis et al, 1985; Price, 1974; Prouty, 1977). Cocaine tissue distribution in cases where psychiatric drugs were also detected has not been widely reported in the literature. Blood cocaine concentrations detected in a series of such cases, however, are shown in Table 1.13 (Fu et al, 2000; Goeringer et al, 2000a). In one case, cocaine was detected in combination with citalopram in femoral and heart blood (0.03 and 0.02 mg/L, respectively), as well as brain (0.1 mg/L) and liver (0.05 mg/L) (Fu et al, 2000).

1.5.4.3. Benzodiazepines

Benzodiazepine use is associated with wide-ranging effects, including restlessness, hallucinations, and hypomanic behaviour. Some users display bizarre, uninhibited behaviour or exhibit hostility, irritability, and rage. These sometimes seemingly paradoxical behaviours cause this class of drugs to figure prominently in cases of assault, sexual abuse, and homicide (Drummer, 2001). Paranoia, depression, and suicidal ideation are also often observed in patients using benzodiazepines (Jonas et al, 1992; Rothschild, 19992).

Benzodiazepines have been used medically as anxiolytics, antidepressants, muscle relaxants, and anticonvulsants, for pre-operative sedation, and to treat acute alcohol withdrawal and panic

attacks (Drummer, 2001). Despite the wide range of applications for drugs belonging to this class, they are quite similar pharmacologically.

Söderpalm and colleagues have shown in a number of reports that anxiolytic effects are observed in rats after destruction of 5-HT neurons and axons with serotonin-depleting compounds, inhibition of 5-HT synthesis, and administration of 5-HT_{1A} receptor agonists which stimulate inhibitory serotonergic autoreceptors on serotonin cell bodies (Soderpalm and Engel, 1989, 1990, 1991; Soderpalm et al, 1997). They proposed this effect may involve release of positive endogenous modulators of the $GABA_A$/benzodiazepine receptor complex, such as GABA, endogenous benzodiazepines, or neurosteroids (Soderpalm et al, 1997). However, recognized differences in drug effect patterns within various animal models suggest distinct serotonin pathways may modulate the different classes of anxiety (Tsuji et al, 2000). It has further been reported that 5-HT axonal terminals make synaptic contacts with cortical and hippocampal GABA interneurons (Freund et al, 1990; Halasy et al, 1992). Sibille et al showed that genetic inactivation of the 5-HT_{1A} receptor results in abnormalities in $GABA_A$ receptor composition and concentration, which in turn results in relative sensitivity to benzodiazepines and development of anxiety (Sibille et al, 2000). Over 90% of the 5-HT_3 receptor-expressing cells in the neocortex and hippocampus are GABAergic, and can affect local GABA release (Morales et al, 1996; Ramirez et al, 1996). In light of these facts, Nazar et al have proposed that decreased serotonin concentrations might enhance the direct facilitating influence of benzodiazepines on activity of hippocampal GABA innervation, thus leading to more potent anxiolytic-like action of benzodiazepines (Nazar et al, 1999).

The anticonvulsant benzodiazepines, which include clobazepam, clonazepam, clorazepate, diazepam, and lorazepam, act by augmenting the inhibitory effects of stimulating GABAergic pathways. GABA-induced changes in membrane potential are also enhanced, probably due to an increased frequency of bursts of openings of GABA-activated Cl channels (Twyman et al, 1989).

The net effect of administration of hypnotic benzodiazepines is to increase total sleep time, largely because of an increase in time spent in stage two, the major fraction of non-REM sleep. Additionally, the number of shifts to lighter sleep stages and the amount of body movement are diminished (Drummer, 2001). However, during chronic nocturnal use the effects on the stages of sleep decline within a few nights (Lader and File, 1987).

At peak concentrations, hypnotic doses of benzodiazepines cause light-headedness, lassitude, increased reaction time, motor incoordination, impairment of mental and motor functions, confusion, and anterograde amnesia. Residual daytime sleepiness may also be present, although use of short acting benzodiazepines reduces this effect (Dement, 1991).

Common side effects associated with benzodiazepine use include weakness, headache, blurred vision, vertigo, nausea, vomiting, epigastric distress, and diarrhoea (Meyer, 1982; Morselli, 1977; Symposium (Various authors), 1982, 1983). Additionally, joint and chest pains and incontinence may occur in some subjects. Anticonvulsant benzodiazepines, such as clonazepam, clorazepate, diazepam, and lorazepam, may actually increase the frequency of seizures in patients with epilepsy. After repeated use, subjects become tolerant to their effects. In these subjects, reducing the dose or stopping the medication produces withdrawal symptoms. It can be difficult,

however, to distinguish withdrawal symptoms from the reappearance of the anxiety symptoms that caused the benzodiazepine to be prescribed in the first place (Hardman and Limbird, 1996).

Withdrawal symptoms may also include dysphoria, irritability, sweating, unpleasant dreams, tremors, anorexia, and faintness or dizziness. The use of high doses of benzodiazepines over prolonged periods can lead to more severe symptoms after discontinuing the drug, including agitation, depression, panic, paranoia, myalgia, muscle twitches, and even convulsions and delirium. In spite of these adverse effects, benzodiazepines are relatively safe drugs (DuPont, 1988; Woods et al, 1992). Even huge doses are seldom fatal unless other drugs, particularly alcohol, are taken concomitantly. Onset of true coma is uncommon in the absence of another CNS depressant, which can lead to death if not treated rapidly. Death may also occur as a complication of unconsciousness, due to respiratory depression with aspiration of gastric contents, or as a result of aspiration pneumonia (Drummer and Ranson, 1996). Therapeutic doses of benzodiazepines may also further compromise respiration in patients with chronic obstructive pulmonary disease or obstructive sleep apnoea.

Few cases of fatal diazepam intoxication have been reported in the literature. In a compilation of 67 fatal cases involving diazepam, Dinovo et al divided the cases into three groups. Blood diazepam concentrations in cases involving other drugs averaged 18 mg/L. In five cases of diazepam-alcohol combined toxicity, the average blood concentration was 5.2 mg/L, and the average blood concentration in three diazepam-only deaths was 4.8 mg/L (Dinovo et al, 1976). In a study of over 1200 diazepam-related deaths, only 2 cases were ascribed solely to the ingestion of diazepam (Finkle et al, 1979). Diazepam has often been detected in combination

with psychiatric drugs in fatalities reported in the literature (Anastos et al, in press; Bidanset et al, 1999; Goeringer et al, 2000a; Singer and Jones, 1997). Like the other drugs of abuse discussed earlier, however, blood diazepam concentrations in such cases were considerably lower than in deaths attributably mainly to diazepam toxicity (Table 1.13).

In postmortem blood specimens from subjects who have taken flunitrazepam, little or no parent drug is usually detected, although high concentrations of the corresponding 7-amino metabolite are found (Drummer and Ranson, 1996; Iten, 1992; Lloyd and Parry, 1989). Drummer et al (Drummer et al, 1993b) found that in a number of cases where flunitrazepam was taken alone, the mean 7-amino flunitrazepam concentration was 0.45 mg/L. When taken in combination with alcohol, the mean concentration was reduced to 0.16 mg/L. In a later report, Drummer & Ranson (Drummer and Ranson, 1996) investigated 16 deaths associated with toxic concentrations of benzodiazepines, 11 of which involved significant pre-existing natural disease. The other 5 deaths were attributed solely to the presence of benzodiazepines. In all cases, flunitrazepam was detected mainly as the 7-amino metabolite. They concluded that flunitrazepam may cause death in the absence of other drugs or significant disease, and that the presence of alcohol reduces the amount of flunitrazepam needed to cause death. At the time of this dissertation, there had been no deaths reported in the literature in which flunitrazepam was detected in the presence of psychiatric drugs.

Deaths involving oxazepam often occur in the setting of natural disease. An ante-mortem blood oxazepam concentration of 2.4 mg/L was measured in a 60-year-old male with a history of emphysema, bronchitis, and heart disease (Drummer and Ranson, 1996). In a similar case, a

postmortem blood specimen taken from a 76 year old male with a history of coronary artery atherosclerosis was found to contain 4.6 mg/L oxazepam (Drummer and Ranson, 1996). As with flunitrazepam, no cases have been published in which oxazepam has been detected in combination with psychiatric drugs.

Twelve fatalities involving temazepam in combination with one or more drugs were reported by Forrest et al in which temazepam blood concentrations ranged from 0.9-14 mg/L (Forrest et al, 1986). Two individuals who had ingested temazepam only had blood and liver drug concentrations of 3.8-9.0 mg/L and 39-107 mg/kg, respectively (Martin and Chan, 1986). The presence of temazepam in blood at concentrations ranging from 0.5-7.8 mg/L has been reported in three psychiatric drug-positive postmortem cases (Table 1.13) (Anastos et al, in press; Goeringer et al, 2000a).

1.5.4.4. Cannabis

The desired physiological effects of cannabis occur in the CNS. These include euphoria, followed by anxiety, paranoia, panic, and generally unpleasant feelings (Drummer, 2001). Sedation is also common after use, but most importantly, cannabis has been shown to cause impaired memory, as well as divided attention, cognition, and perceptual tasks (Ashton, 1999; Dewey, 1986; Jones, 1987; Mason and McBay, 1985). However, smoking cannabis also results in a number of side effects, including increased heart rate, blood pressures and body temperature. It is also known to reduce intraocular pressure and cause lacrimation and vasodilation leading to reddened conjunctiva, and bronchodilatation of the small airways of the lung.

Many of the acute psychological and physiological effects of marijuana are mediated by the CB_1 receptors in animals as well as humans (Darmani, 2001, 2002; Huestis et al, 2001). Studies of the relative cannabinoid receptor affinity and efficacy of Δ^8-THC side-chain analogues have shown that a ligand's CB_1 affinity and efficacy are differentially altered by modifications in the side-chain (Griffin et al, 2001). Δ^9-THC has slightly higher affinity for the CB_1 versus the CB_2 receptor (Pertwee, 1997). Importantly for the purposes of this dissertation, the cannabinoid receptors have been shown to have complex effects on serotonin, dopamine, noradrenaline, and GABA. These effects include inhibition of serotonin and noradrenaline release in rat, guinea-pig, and human brain (Nakazi et al, 2000; Volfe et al, 1985). They include an increase in central cholinergic activity, a moderate increase in catecholaminergic activity, and effects on GABA and dopamine, as well (Spadone, 1991).

Smoking cannabis has been linked to a 20 % decrease in the striatal dopamine D_2 receptor binding ratio, suggesting increased synaptic dopaminergic activity and possibly underlying the reinforcing and abusing properties of marijuana (Ameri, 1999; Voruganti et al, 2001). Cannabinoids have also been shown to modulate GABAergic synaptic transmission, which may explain some of the associated emotionally relevant behavioural effects of exposure (Katona et al, 2001). Finally, they have been shown to moderate the antinociceptive activity of opioids.

The antianxiety effect of Δ^9-THC is mediated through central $GABA_A$/benzodiazepine receptors (Sethi et al, 1986). Voruganti et al postulate that the psychotogenic effects of cannabis in certain subjects are a result of increased synaptic dopaminergic activity after smoking (Voruganti et al, 2001). These effects appear to irreconcilably link the dopamine D_2 and cannabis CB_1 receptors.

Fritzsche found that CB_1 knockout mice exhibit behavioural changes that mimic symptoms of schizophrenia, cannabis intoxication, and dopamine D_2 activation (Fritzsche, 2001). He suggested the CB_1 receptor is responsible for the varied aspects of schizophrenic psychosis.

Common side effects associated with heavy cannabis use include panic attacks, hallucinations, and muscle tremor. Acute paranoid or manic psychosis may occur as a result of cannabis intoxication. These events consist of paranoid ideation, illusions, hallucinations, delusions, depersonalisation, confusion, restlessness, and excitement (Mathers and Ghodse, 1992). Epidemiological studies show an increase in incidence of schizophrenia among cannabis users. Subjects known to have used cannabis more than 50 times are six times more likely to develop schizophrenia than non-users (Andreasson et al, 1987).

Other side effects include changes in the menstrual cycle in women and decreased sperm count and impaired sperm motility with abnormal morphology in men (Drummer, 2001). Long-term marijuana smoking on a daily basis has been shown to precipitate pulmonary dysfunction and may lead to chronic obstructive lung diseases.

Cannabis use, particularly smoking, has rarely been found to be the direct cause of death. In one case, however, in which a man was believed to have died of an acute overdose after oral ingestion of cannabis. THC concentrations in liver and kidney were 38 and 42 mg/kg, respectively (Tewari and Sharma, 1980). Cannabis and metabolites can be measured at surprisingly high concentrations in living subjects. Urine 11-carboxy-THC concentrations up to 2705 µg/L have been observed in frequent users (Baselt, 1984), and even passive inhalation of

marijuana smoke has resulted in urine 11-carboxy-THC concentrations as high as 39 µg/L and plasma THC concentrations of 1-7 µg/L (Cone and Johnson, 1986; Cone et al, 1987; Mason et al, 1983; Morland et al, 1985).

Cannabis is frequently associated with traffic crashes. Data is emerging suggesting it increases the risk of traffic crash causation (Chu, 2002; Fergusson and Horwood, 2001). During a one-year period, Δ^9- THC was present in the blood of 10 out of 159 victims of fatal auto accidents at concentrations ranging 4-14 µg/L (McBay, 1981). In 66 similar blood specimens tested using radioimmunoassay, 6 were positive for cannabinoids, 3 of which contained Δ^9-THC at concentrations between 2-4 µg/L (Law, 1981; Teale et al, 1977). Although often detected in psychiatric drug-present deaths using immunoassay techniques, Δ^9-THC is often not quantitated in such cases.

1.5.4.5. Opioids

The primary effects of opioids are on the central nervous system. When administered therapeutically in patients suffering from pain, opioids reduce both the sensation of pain and distress. However, subjects may experience nausea, vomiting, drowsiness, lethargy, and reduced physical activity. Patients may also experience euphoria and respiratory depression.

These adverse effects are likely in subjects suffering from pain. Injection of a heroin solution, however, produces a variety of sensations described as warmth, taste, or a "rush", which has led to its extensive abuse in street users.

Opioids exert effects on dopamine, GABA, noradrenaline, and serotonin systems in addition to their effect on opioid receptors. Spinal 5-HT-containing neurons, for example, play a crucial role in the development of morphine tolerance (Li et al, 2001). Mikkola and colleagues have linked the acute locomotor stimulatory effects of morphine to effects of both dopamine and serotonin in rat caudate-putamen, nucleus accumbens and olfactory tubercle (Mikkola et al, 2001). Opioid effects on noradrenaline have also been observed. Opioid-induced vertigo has been linked to elevated plasma adrenaline concentrations, although no such link with noradrenaline concentrations has been found (Hogger, 2000). Gadek-Michalska et al have published a study suggesting interaction of opioids with central α_2- but not α_1-adrenoceptors (Gadek-Michalska et al, 1997; Gray et al, 1999). In this study, naloxone administered intracerebroventricularly to conscious rats was observed to significantly decrease the corticosterone response to noradrenaline (which stimulates the hypothalamic-pituitary-adrenal axis via α_2-adrenoceptors) but not adrenaline (which acts mainly using α_1- and β-adrenoceptors). Another study in which both endogenous and exogenous opioid-mediated analgesia were potentiated by elimination of the noradrenaline transported in mice provides further support for opioid interaction with central α_2-adrenoceptors (Bohn et al, 2000).

Morphine and related opioids must be used cautiously in patients with compromised respiratory function, including subjects with emphysema, kyphoscoliosis, or severe obesity. Opioids occasionally precipitate asthma attacks in anaesthetised patients (Hardman and Limbird, 1996). Subjects who take an overdose of an opioid is usually stuporous or may be in a profound coma. Respiratory rate will be very low and cyanosis may be present. If hypoxia persists, there may be

capillary damage and measures to combat shock may be required. Pupils will be symmetrical and pinpoint in size unless hypoxia is severe, in which case pupils will be dilated.

The largest numbers of deaths due to opioid toxicity have been attributed to acute toxicity shortly after injection of heroin or morphine (Baden, 1980; Helpern, 1972). There are three main mechanisms:

   a) death from profound respiratory depression

   b) death from cardiovascular collapse

   c) death as a consequence of severe pulmonary oedema (Cotran et al, 1989)

Mechanisms a) and b) occur as a result of the shallow respiration typically exhibited by subjects who experience heroin toxicity (Shuckit and Segal, 1995). If a subject becomes unconscious, the airway may be obstructed and respiratory function diminished. As a result, pulmonary oedema and/ or congestion occurs.

Even if respiration is restored, death still may occur as a result of complications that develop during the period of coma, such as pneumonia or shock (Ostor, 1977). Noncardiac pulmonary oedema is the most common pathological finding in autopsies of victims of opioid toxicity, and has been observed following toxic doses of morphine, methadone, propoxyphene, and uncontaminated heroin (Cotran et al, 1989).

Following the dramatic increase in mortality amongst intravenous drug users in the early 1970's,

Richards et al (Richards et al, 1976) assessed tissue distribution patterns of heroin and morphine

in 114 cases where death resulted from intravenous administration. Deaths were categorized as

either a) heroin only, b) heroin and alcohol deaths, or c) heroin and other drug deaths. Although

the concentration ranges and means varied between the three groups, the median value was fairly

constant for each group (group A: 0.06 mg/L, group B: 0.05 mg/L, and group C: 0.04 mg/L).

The presence of opioids, particularly morphine and codeine, has frequently been reported in

combination with psychiatric drugs in the literature. In such cases, blood and urine

concentrations are often reported, although concentrations in other tissues usually are not. As

shown in Table 1.13, these concentrations often overlapped with those seen in the three

categories of heroin-related deaths described by Richards et al (Richards et al, 1976).

1.6. Brain distribution of antipsychotic drugs in postmortem cases

1.6.1. General brain physiology

There are four main regions of the CNS, each with a distinct, designated function: the spinal

cord, the brain stem, the diencephalon, and the cerebral hemisphere (Figure 1.18). The spinal

cord is divided into cervical, thoracic, lumbar, and sacral regions, while the brain stem is

comprised of the medulla oblongata, pons, cerebellum, and midbrain. The thalamus and

hypothalamus make up the diencephalon, and the cerebral hemisphere consists of the cerebral

cortex, the basal ganglia, the hippocampus, and the amygdaloid nucleus. Known functions of

each of these regions are shown in Table 1.14.
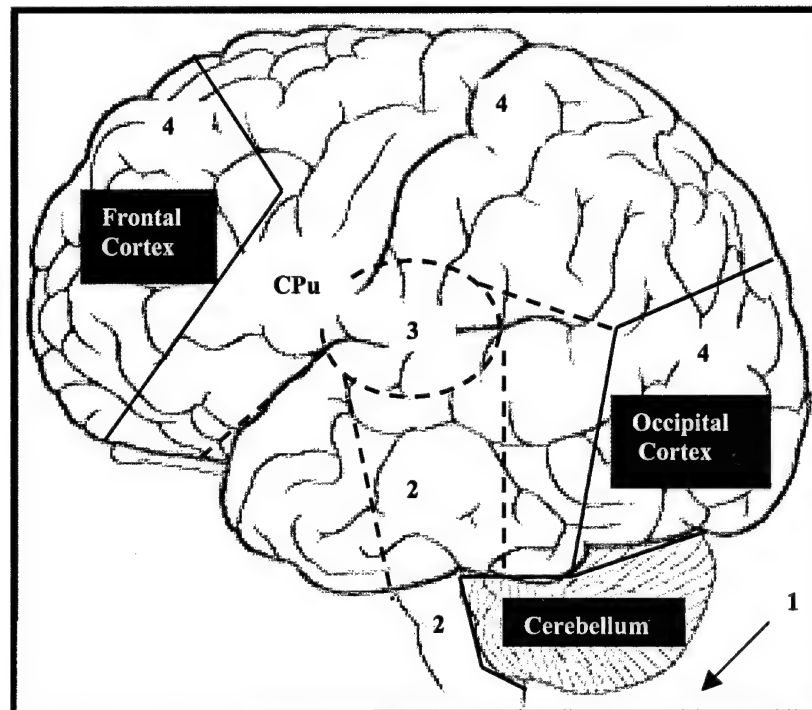
Figure 1.18. Major CNS regions: 1) spinal cord, 2) brain stem, 3) diencephalon, and 4) cerebral hemisphere; and brain regions with believed involvement or non-involvement in schizophrenia.

Table 1.14, Functions of the main parts of the CNS (after Kandel)[1].

Region	Subregion	Function
Spinal Cord	N/A	controls movement of limbs and trunk
Brain Stem	Medulla oblongata	control of vital autonomic functions (digestion, breathing, control of heart rate)
	Pons	conveys movement information from cerebral hemisphere to cerebellum
	Cerebellum	modulates force & range of movement; involved with learning of motor skills
	Midbrain	controls sensory & motor functions (eye movement, coordinating visual & auditory reflexes)
Diencephalon	Thalamus	processes information from rest of CNS to cerebral cortex, location of Wernicke's area
	Hypothalamus	regulates autonomic, endocrine, & visceral function
Cerebral hemispheres	Cerebral cortex	initiation and processing of cognition (language, thought, reading, hearing, speech)
	Basal ganglia	regulates motor performance, approximate location of Broca's area
	Hippocampus	involved with aspects of memory storage
	Amygdaloid nucleus	coordinates autonomic and endocrine responses w/ emotional states

[1] (Kandel, 1991)

The cerebral cortex is divided into four lobes: the frontal, parietal, temporal, and occipital. These regions are involved in cognition in varying ways, depending on the type of mental processing required. Brodmann further subdivided the cerebral cortex into 52 discrete areas based on cell structure and arrangement (Garey, 1994). Brodman's divisions have come to be commonly used in the discussion of brain function, and have been investigated to a great extent to understand how the brain performs different types of mental processing. Wernicke's area, near what is known as the primary auditory cortex, for example, combines auditory input with information from other senses. Another example is Broca's area, lying near the vocalization region of the

motor area, which issues specific commands that cause the mouth and tongue to form words (Geschwind, 1974). Although their functions are discrete, these regions have been discovered to act in concert with each other depending on the type of action.

1.6.2. Behavioural and neuropathological aspects of schizophrenia

According to the *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)*, schizophrenia is a disorder which lasts at least six months and includes a month or more of at least two of the active-phase symptoms listed in Table 1.15 (American Psychiatric Association, 1994). Schizophrenia can be thought of as a mental disorder with two dimensions. The psychotic dimension consists of delusions and/or hallucinations, while the disorganisation dimension involves disorganised speech and behaviour. The disorder can be further categorized as either schizophrenoform (shorter duration, i.e. 1-6 months, with no requirement for declined functioning), schizoaffective (mood episode and symptoms occur together, preceded or followed by at least two weeks of delusions or hallucinations without prominent mood symptoms), delusional (one month of non-bizarre delusions without other symptoms of schizophrenia), brief psychotic (lasting more than one day but gone within one month), or shared psychotic (delusions occurring in someone who has been in contact with someone who exhibits active-phase symptoms) (American Psychiatric Association, 1994).

Table 1.15. Active-phase symptoms of schizophrenia[1].

Positive Symptoms[2]	Negative symptoms[2]
distortions in thought content (delusions)	restrictions in range/intensity of emotional expression (affective flattening)
distortions in perception (hallucinations)	restrictions in fluency/productivity of thought & speech (alogia)
distortions in language/thought process (disorganised speech)	restrictions in initiation of goal-directed behaviour (avolition)
distortions in self-monitoring behaviour (grossly disorganised/catatonic behaviour)	

[1] (American Psychiatric Association, 1994). [2] Positive symptoms involve excesses or distortions of normal function, while negative symptoms involve diminution or loss of normal functions.

Recent studies on the neuropathology of schizophrenia have highlighted structural alterations in the brains of schizophrenic patients (Halliday, 2001). Most commonly cited features in chronic schizophrenics are diffuse ventricular enlargement and decreased cortical volume (Halliday, 2001; Schmitt et al, 2001; Steel et al, 2002). These alterations are thought to be neurodevelopmental in origin, as they have been observed in subjects in so-called "first-episode cases", i.e. those with no prior history of schizophrenia (Lawrie et al, 2001). However, a more detailed subdivision of the brain shows this reduction in volume is limited to specific brain regions. Within the frontal cortex (see Figure 1.18), 70% of studies using non-invasive morphologic imaging showed a decrease in overall volume, while 63% noted a reduction in size in the thalamus and 60% in the cerebellum (Schmitt et al, 2001). The caudate-putamen is another area of the brain involved with schizophrenia for which decreased structural volumes have been

observed (Lang et al, 2001). Based on what is known about brain physiology, it is likely the concerted functioning of multiple regions is affected in this disease. Phenotypic changes in schizophrenia at the cellular level, however, have yet to be identified, and the relationship between neurochemical observations and these structural changes is yet to be understood.

1.6.3. Antipsychotic drug partitioning in the schizophrenic brain

Svendsen et al wrote what appears to be the only published study to report postmortem concentrations of thioridazine and its metabolites and chlorpromazine in selected brain regions from schizophrenics (Svendsen et al, 1988b). The regions studied were frontal cortex, motor cortex, cingulate cortex, temporal cortex, caudate nucleus, caudate putamen, thalamus, amygdala, hippocampus, substantia nigra, red nucleus, cerebellum cortex, and cerebellum deniale. A review of the clinical histories revealed that 5 subjects had received thioridazine up to 8 hours or less before death. In these subjects, no one area of the brain selectively accumulated thioridazine or its metabolites after chronic treatment. One of the remaining subjects, however, who had been withdrawn from medication 4 days before death showed more specific parent drug and metabolite distribution (see Table 1.16) (Svendsen et al, 1988b). The most remarkable difference was between cortical and subcortical structures, in which the former had high thioridazine levels and lower mesoridazine levels, whereas the latter had high mesoridazine and lower thioridazine concentrations. Aravagiri and van Beijsterveldt published similar results for fluphenazine and risperidone in rats, showing highest concentrations for both drugs in cortical structures (see Table 1.16) (Aravagiri et al, 1995; van Beijsterveldt et al, 1994).

Merrick and colleagues published a study on the tissue distribution of olanzapine in a postmortem case in which they also measured drug concentrations in seven regions of the brain (see Table 1.16) (Merrick et al, 2001). They reported highest concentrations in midbrain, followed by caudate-putamen, right frontal cortex, hippocampus, left frontal cortex, and amygdala. Analysis of cerebellum returned negative results.

One study of plasma concentrations of thioridazine and metabolites (Cohen et al, 1989) in humans found that although drug levels varied widely between patients, in all patients the relative level of thioridazine to mesoridazine was about one half and thioridazine to sulforidazine was two-fold. If the ratio of parent drug to metabolite in brain is similar to that of blood, information regarding regional brain distribution of antipsychotics may be inferred from studies which used plasma or blood concentrations. However, a study of antipsychotic drug distribution between blood and whole brain in rats seems to indicate this is unlikely, (Tsuneizumi et al, 1992). The observed ratio of brain-to-serum concentration varied widely, and the high potency drugs fluphenazine and haloperidol had higher brain-to-blood distribution than the lower potency thioridazine.

Table 1.16. Summary of published data on regional brain distribution of selected antipsychotics[1].

Reference	Drug	CPu/Striatum	FCx[2]	Hippocampus	Midbrain	Amygdala	Cerebellum
(Aravagiri et al, 1995)	fluphenazine	1300	2500	1800	1700	--	1200
(Merrick et al, 2001)	olanzapine	390	170 L, 370 R	220	860	160	negative
(Svendsen et al, 1988)	thioridazine	0.002	0.004	0.001	0.001		
(van Beijsterveldt et al, 1994)	risperidone	6.9	7.6	--	--	--	1.4

[1] All concentrations in ng/g. Highest regional concentrations in red, lowest in blue. [2] Cortex in studies of rat brain; L = left, R = right.

1.7. Redistribution of monoamine-active drugs in postmortem cases

An occurrence known as postmortem redistribution, in which drug concentrations in blood specimens from various areas of the body change during the postmortem interval prior to autopsy has been observed for many drugs (Bandt, 1980; Barnhart et al, 2001; Jones, 1985; Prouty and Anderson, 1984, 1986). In a review of this subject, Prouty and Anderson (Prouty and Anderson, 1989) discussed several reports in which the central blood concentration of a particular drug was significantly higher than that of peripheral blood. Specimens from multiple sites were analysed, and the authors concluded that no one collection site consistently produced the highest concentration, and that it is impossible to predict which specimen will exhibit the largest change in drug concentration over time.

Bandt showed TCAs may undergo postmortem redistribution in a study where blood samples were taken from a number of sites as well as serially from the same site (Bandt, 1980). He concluded that TCA concentration generally increased with time and that the drug concentration was a function of the origin of the blood specimen. Bandt suggested the process of postmortem redistribution might be driven by diffusion via a concentration gradient in which drugs are released from the liver and drug-rich liver blood, then diffuse into the larger vessels and subsequently into the right atrium. Drugs with high volumes of distribution, i.e. greater than 3 L/kg, are thus more prone to postmortem redistribution.

Several theories have been proposed to explain the phenomenon of postmortem redistribution. The first follows Bandt's reasoning, that postmortem, tissue and muscle sites known to

accumulate a drug at high concentration release the drug into blood at those sites. Considerable

blood movement postmortem, due to the build-up of gases produced through decomposition

allows for mixing of blood from various sites, so drug concentrations in blood specimens taken

from vessels near or related to the major organs may not reflect the actual blood concentration

(Prouty and Anderson, 1990). For these reasons, blood collected peripherally is thought to most

accurately reflect the true drug concentration (Pounder et al, 1996a; Prouty and Anderson, 1990).

Another possible explanation for redistribution is that the administered drug may be incompletely

distributed prior to death. Baud et al reported an amitriptyline overdose which appears to support

this theory (Baud et al, 1985). This may also occur if unabsorbed drug diffuses postmortem from

the gastrointestinal tract into neighbouring tissues.

An understanding of postmortem tissue distribution is important to determine the role of these

drugs in the death process. Tissue distribution of several SSRIs and atypical antidepressants has

been studied (Bidanset et al, 1999; Budd and Anderson, 1996; Jaffe, 1997; Levine et al, 1996;

Logan et al, 1994; Parsons et al, 1996; Vermeulen, 1998). In many cases, only one blood sample

was taken, so it was impossible to determine if redistribution had occurred. The high volumes of

distribution of SSRIs (3-28 L/kg) and antipsychotics (1.2-1000 L/kg) make postmortem

redistribution likely, but it is a subject only beginning to be studied with regard to these drugs.

In cases where both central and peripheral blood specimens were taken in the mentioned tissue

distribution studies, no significant difference was seen for sertraline (Jaffe, 1997; Logan et al,

1994) (see Table 1.17).

Table 1.17. Concentrations of psychiatric drugs in central and peripheral blood [1].

Reference	Blood Concentrations		Ratio (Cen:Per)
	Central (mg/L)	Peripheral (mg/L)	
Citalopram			
(Fu et al, 2000)	1.16	0.88	1.32
Fluoxetine			
(Jaffe, 1997)[2]	0.78-0.80 (0.42-0.70)	0.65 (0.53)	1.2 (0.79-1.3)
(Rohrig and Prouty, 1989)	0.80 (0.65)	0.23 (0.25)	3.5 (2.6)
(Roettger, 1990)	22 (6.8)	4.8 (4.5)	4.6 (1.5)
Fluvoxamine			
(Kunsman et al, 1999)	1.5	0.48	3.1
Paroxetine			
(Jaffe, 1997)[2]	0.10-0.18	0.12	0.83-1.5
(Vermeulen, 1998)	3.7	2.9	1.28
Risperidone – no data			
Sertraline			
(Jaffe, 1997)[2]	0.38-0.48	0.25	1.5-1.9
(Levine et al, 1994)	0.23-0.46 (0.08-0.99)	0.23-0.82 (0.17-1.8)	0.56-1.36 (0.55-1.77)
(Logan et al, 1994)	0.49 (1.40)	0.63 (1.54)	1.29 (1.10)
Venlafaxine			
(Jaffe, 1997)[2]	1.0-1.5	0.5	2.0-3.0
(Levine et al, 1996)	84 (15)	46 (7.1)	1.83 (2.11)
(Parsons et al, 1996)	17-65 (7.1)	30-85 (5.6)	1.31-1.76 (0.79)

[1] Metabolite concentrations listed in parentheses. [2] Autopsy:admission bloods instead of heart:femoral.

Slight differences in paroxetine and fluoxetine concentrations were observed in central versus peripheral blood in one case of a subject who died in hospital (Vermeulen, 1998), but neither drug was present on admission. It was suggested that the drugs were administered in hospital and the differences in concentration between the two specimens were most likely a result of incomplete absorption. In the two studies in which postmortem peripheral and central blood

venlafaxine and metabolite concentrations were compared, central blood concentrations were approximately 1.5-2 times those found in peripheral blood (Jaffe, 1997; Levine et al, 1996; Parsons et al, 1996).

1.8. Methods for detecting monoaminergic drugs and their metabolites

The high pK_a's of monoamine-active drugs mean they become completely deprotonated only at pH's well above 7.0. Because of the likelihood of the presence of multiple drugs in subjects taking psychiatric medications, it is desirable to develop a separation and detection method capable of separating all drugs in one chromatographic run. From an analytical standpoint, the possibility of multiple drugs present in an individual means chromatographic separation of such a complex drug mixture may be difficult to achieve. Often, laboratories use specific methods for small subsets of drugs with similar properties. It would be desirable to perform screening and confirmation in one analysis, as it would result in savings of both time and money.

The problem of analysing monoamine-active drugs has plagued toxicology laboratories for many years. Their generally high polarity makes them difficult to separate using gas chromatography without derivatisation. The basicity of most monoamine-active also makes their analysis by HPLC difficult due to the limited operating pH range of conventional HPLC columns.

Commercially available reversed-phase silica-based columns fail at high pH as a result of solubilization of the silica support, reducing their potential lifetime (Vervoort et al, 1992;

Vervoort et al, 1994). A number of researchers have explored stability of newer stationary phases in HPLC analysis of basic drugs (Kele and Guichon, 1999a, 1999b, 1999c, 2000; Li et al, 2000; Needham et al, 1999; Needham et al, 2000). Kele and Guiochon studied column-to-column and batch-to-batch reproducibility of retention data on the following monomeric chemically bonded silica columns: Symmetry C_{18} (Waters) (Kele and Guichon, 1999c), the Kromasil C_{18} (Eka) (Kele and Guichon, 1999a), and the Luna C_{18} (Phenomenex) (Kele and Guichon, 2000). They measured retention time reproducibility for selected acidic, neutral, and basic compounds using unbuffered mobile phases as well as phases buffered from pH 2.7 to pH 7.0. They found that the relative standard deviations (RSDs) of retention times of all compounds measured on each of the five columns consistently increased with increasing retention time. Additionally, they observed that the RSD figures for neutral and acidic compounds are much lower than those for basic compounds at acidic and neutral pH.

To compound this problem, drug metabolites are often pharmacologically active and hence contribute to the effect of the drug, but are also structurally similar to the parent compounds. Atypical antidepressants such as venlafaxine or antipsychotics like risperidone are prime examples. The structural similarity of these compounds to the parent drugs leads to similar chemical and physical characteristics, which in turn makes chromatographic separation more difficult. Moreover, blood, urine, and other complex biological matrices are comprised of many endogenous compounds that may interfere with the quantitation of drug levels.

1.8.2. Sample preparation methods

1.8.2.1. Liquid-liquid extraction

In most cases, chromatography requires some sort of sample clean-up procedure to separate the drugs from the biological matrix. The most common types of procedures fall into the category of either liquid-liquid or solid-phase extraction. Liquid-liquid extraction has long been the accepted approach to drug isolation from a biological matrix, although many of the broad-spectrum screening method published over the last decade, especially those which use GC, involve the use of solid-phase extraction (Drummer, 1999). Liquid-liquid extraction methods typically employ the use of polar organic solvents from an alkaline buffered aqueous solution (Derendorf and Kaltenbach, 1986; Foerster and Mason, 1974; Foerster et al, 1978; Logan et al, 1987; McIntyre et al, 1993b; Polettini et al, 1998). Buffer pH's have been in the range of 7.0-10.0. Extraction methods for drug of abuse determination have been reviewed in-depth by Wilson et al (Wilson et al, 2001). For both drugs of abuse and psychiatric drugs, butyl chloride and hexane have been commonly used as extraction solvents (Drummer et al, 1994). Hexane or chloroform with modifiers have also been used to extract specimens suspected of having multiple psychiatric drugs (Decaestecker et al, 2000; Maurer and Bickeboeller-Friedrich, 2000).

1.8.2.2. Solid-phase extraction

Solid-phase extraction (SPE) methods have become more popular in recent years due to a number of factors, including lower cost due to reduced solvent/reagent consumption (Gerastomoulos, 1997), greater drug recoveries particularly for opioids (Crump et al, 1994; Felby et al, 1974; Logan et al, 1987) and benzodiazepines (Chen et al, 1993), reduced sample preparation time, and reduced possibility for introduction of human error due to less sample handling. Solid-phase extraction supports have been made using XAD-2 resin and diatomaceous earth. Recent advances in extraction column technology have led to availability of various supports made of organosilanes with a variety of functional groups (C_2, C_8, C_{18}, CN, and phenyl) to suit particular analytical needs. Additionally, a number of proprietary mixed-phases such as Bond-Elut Certify, Chromabond, Isolute HCX, TSC and CleanScreen DAU (Drummer, 1999) have further improved the separation power and recovery efficiency of solid-phase extraction. Mixed-phase extraction columns show good recoveries and allow retention of compounds possessing a variety of functional groups and differing polarities. Specimen preparation using SPE has become common in drug of abuse screening methods, the most commonly used being the Bond-Elut Certify and Bakerbond cartridges (Paterson et al, 2000; Rittner et al, 2001; Soriano et al, 2001). However, SPE has yet to see extensive use in psychiatric drug analyses.

1.8.3. Sample analysis methods

1.8.3.1. Immunoassay

Toxicological analysis of monoaminergic drugs is traditionally carried out in two phases: screening via enzyme multiplied immunoassay techniques (EMIT™, Syva) followed by confirmation, either by GC or HPLC. EMIT allows direct analysis of samples without the need for extraction. In this technique, analytes of interest in the specimen compete with synthetic copies of the analyte to which has been added a signal producing label for antibody binding sites. Thus, higher drug concentrations in the specimen give rise to a smaller signal. The major drawback with immunoassays is that they are non-specific in their binding, resulting in cross-reactivity among several drugs in a particular drug class (opioids, amphetamines, etc). Furthermore, reactivity can be affected by variations in pH, ionic strength and the presence of other chemical substances (Gerastomoulos, 1997). Despite these problems, immunoassay can be used quite successfully for screening when followed by confirmation via HPLC or GC.

1.8.3.2. Gas chromatography (GC)

Gas chromatography, either by itself or coupled with mass spectrometry (MS), has seen extensive use in the toxicology laboratory due to its high specificity and the broad range of detectable compounds. Its use, however, often requires clean-up procedures and in some cases derivatisation or hydrolysis due to polarity and/or thermal lability, particularly in the case of

acidic drugs (Drummer, 1999). By contrast, many basic and neutral drugs can be chromatographed without derivatisation using either non-polar or low-polarity capillary columns (see Table 1.18). Several methods for the determination of drugs of abuse using GC with either nitrogen/phosphorus detection (NPD) or MS have been published, although at least some derivatisation is often required in order to elute drugs belonging to all classes (Girod and Staub, 2000; Karacic and Skender, 2000; Paterson et al, 2000; Soriano et al, 2001; Weinmann et al, 2000b). Because of the differences in properties observed for different drugs of abuse, some methods focus on particular classes. For example, benzodiazepines were not included in three methods (Girod and Staub, 2000; Karacic and Skender, 2000; Weinmann et al, 2000b).

Although the SSRIs can be detected without derivatisation using GC-MS, thermal degradation of some compounds at injection port temperatures has been noted for this class of drugs (Goeringer et al, 2000a). However, good recoveries have been achieved by compound acetylation prior to analysis by GC-MS (Maurer and Bickeboeller-Friedrich, 2000). It should be noted, however, that neither of these methods are able to detect all of the psychiatric drugs listed in Table 1.4.

Table 1.18. Summary of selected GC methods for the separation of basic drugs.

Reference	Specimen, Amount	Drugs Detected	LOD[1] (ng/mL)	LLE[2]	SPE[3]	Derivatising Agent(s)[4]	Detection[5]	Drug Recovery
(Brooks and Smith, 1991)	plasma, 2 mL	13 basic, neutral, & weakly acidic drugs		dichloromethane/acetone	--	none	MS	>75%
(Chen et al, 1992)	plasma, 2 mL	barbiturates, benzodiazepines, stimulants, opioids	not stated	--	Bond Elute Certify	none	FID	82-105%
(Crifasi et al, 1997)	various tissues,	fluoxetine, norfluoxetine	12.5	n-butyl chloride	--	none	MS	not stated
(Dawling et al, 1990)	blood/serum/plasma, 1 mL	more than 40 basic drugs	≤50	n-butyl acetate	--	none	NPD	not stated
(Drummer et al, 1994)	blood/plasma, 1 mL	acid, neutral, & basic drugs	20-50	n-butyl chloride	--	none	FID/NPD	14-100%
(Girod and Staub, 2000)	hair, not specified	opioids, designer amphetamines	0.05-0.2	--	Isolute HCX	PA	MS	71-103%
(Goeringer et al, 2000)	blood, 1 mL	SSRIs	10	n-butyl chloride	--	none	EI-MS	not stated
(Huang et al, 1996)	liver, 0.4 mL	acidic, neutral, some basic drugs	not stated	--	Bond Elute Certify	none	FID, NPD	70-102%
(Karacic and Skender, 2000)	urine, 2 mL	stimulants, opioids, THCCOOH, metabolites	3-12	--	Bond Elute Certify & Certify II	HFBA, PFPA, TFAA, BSTFA	EI-MS	not stated
(Long et al, 1997)	blood, ?	venlafaxine, O-desmethylvenlafaxine	not stated	n-butyl chloride	--	none	NPD	not stated

Reference	Specimen, Amount	Drugs Detected	LOD[1] (ng/mL)	LLE[2]	SPE[3]	Derivatising Agent(s)[4]	Detection[5]	Drug Recovery
(Maurer and Bickeboeller-Friedrich, 2000)	urine, 5 mL	SSRIs	≤ 100	dichloromethane/ isopropanol/ ethyl acetate	--	AA/P	EI-MS	40-80%
(Paterson et al, 2000)	urine, 4 mL	barbiturates, benzodiazepines, stimulants, opioids	50-200		Bakerbond Narc-2	MBTFA	MS	32-110%
(Sims et al, 1991)	various tissues, 200 µL	range of basic & neutral drugs	not stated	diethyl ether	--	none	ECD	70-102%
(Soriano et al, 2001)	various tissues, 1-2.5 mL	barbiturates, benzodiazepines, stimulants, opioids	8-108	--	Bond Elute Certify	BSTFA/TMCS	NPD	61-120%
(Weinmann et al, 2000b)	serum, 1 mL	stimulants, selected opioids	0.4-17	--	Chromabond Drug	TFAA	MS	>85%

[1] LOD = limit of detection. [2] LLE = liquid-liquid extraction. [3] SPE = solid-phase extraction. [4] TFAA = trifluoroacetic acid, PA = propionic anhydride, BSTFA/TMCS = N,O-bis(trimethylsilyl)trifluoroacetamide/ trimethylchlorosilane, MBTFA = N-methylbis(trifluoroacetamide), AA/P = acetic anhydride/pyridine. [5] FID = flame ionization detection, NPD = nitrogen-phosphorous detection, MS = mass spectrometry, ECD = electron capture detection.

1.8.3.3. High-performance liquid chromatography (HPLC)

A large number of papers on HPLC procedures published since 1989 describe applications of systematic toxicological analysis or are capable of detecting a range of drugs, many of which target basic drugs (see Table 1.18). Three of these methods specify use of gradient elution, although mobile phase constituents vary greatly. Choice of column also varies between the methods, including octadecylsilane-based phases, as well as C_8, C_{18}, and CN-bonded phases. One author evaluated several columns (Koves, 1995). The use of microbore columns reduces both analysis time and amount of mobile phase required, but has yet to see widespread use in the toxicology laboratory.

All these procedures employ either photodiode array detection or multi-wavelength scanning, which enables spectral matches to library entries to be made, facilitating drug identification. Although the use of HPLC eliminates the need for derivatisation, coupling this technique with ultraviolet detection greatly reduces detection sensitivity compared to MS, as can be observed in the LOD's listed in Table 1.19 compared to those in Table 1.18. Depending on the therapeutic range of the drug of interest, particularly potent drugs may not be detected unless present at highly toxic concentrations.

Table 1.19. Summary of selected HPLC methods for the separation of basic drugs.

Reference	Specimen, Amount	Drugs Detected	LOD[1]	LLE[2]	SPE[3]	Detection[4]	Drug Recovery
(Drummer et al, 1993)	blood, 250 μL	acid, neutral, & basic drugs; gradient	0.1-5	protein precipitation w/ acetonitrile	--	DAD	27-100%
(Koves and Wells, 1992)	blood, 2 mL	119 acidic, neutral, & basic drugs; isocratic	0.1	toluene	--	DAD	34-91%
(Kristoffersen et al, 1999)	plasma/blood, 0.5 mL	citalopram, fluoxetine, paroxetine, metabolites	0.05-5.0 μmol/L	--	IST C$_8$	fluorescence detection	38-114%
(Lambert et al, 1995)	various tissues, 1 mL or 1 g	basic drugs; gradient	not stated	hexane/ethyl acetate	--	DAD	<10% to >70%
(Logan et al, 1990)	urine, 1 mL	100 basic drugs; gradient	0.05-0.1	--	Bond Elute SCX	DAD	72-86%
(McIntyre et al, 1993)	blood, 1 mL	17 antidepressants & metabolites; isocratic	0.05	n-butyl chloride	--	DAD	≥60%
(Tracqui et al, 1995)	blood/plasma, 2 mL	311 pharmaceuticals, toxicants, & drugs of abuse, isocratic	0.003-0.113	chloroform/2-propanol/ n-heptane	--	DAD	acidic: 20-50% remainder: >60%

[1] LOD = limit of detection, expressed in mg/L or mg/kg, unless otherwise specified. [2] LLE = liquid-liquid extraction. [3] SPE = solid-phase extraction. [4] DAD = diode array detection.

Another drawback of conventional HPLC is limited selectivity. The ultraviolet spectra of many compounds are quite similar, especially for multiple drugs belonging to the same class. This may result in miss-identification, particularly if two drugs coelute in a chromatographic run. Because of this, many methods focus on the analysis of only one compound or a small group of compounds, particularly in the case of psychiatric drugs. Eap and Baumann have reviewed analytical methods for the separate determination of SSRIs (Eap and Baumann, 1996). McIntyre et al published an HPLC-DAD method for the detection of a variety of older antidepressants commonly found in forensic cases (McIntyre et al, 1993a). Like the GC methods discussed in the previous section, however, it is not able to detect all of the psychiatric drugs listed in Table 1.4.

1.8.3.4. Liquid chromatography-mass spectrometry (LC-MS)

LC-MS is a technique which has only recently begun to see widespread use in toxicology laboratories, but which has been reviewed extensively in recent years (Hoja et al, 1997b; Marquet and Lachatre, 1999; Marquet, 2002; Maurer, 1998; Pichini et al, 1999; van Bocxlaer et al, 2000). It provides the separation power of HPLC combined with the increased sensitivity and selectivity of MS (Hoja et al, 1997a; Maurer, 1998). In contrast to the conventional HPLC methods detailed in Table 1.15, most of the LC-MS methods used gradient elution (Table 1.20). This may be due to the fact that the baseline drift, which interferes with quantitation in HPLC analyses using ultraviolet detection, is not observed in LC-MS when selected ion monitoring is used.

Table 1.20.   Summary of selected LC-MS methods for the separation of psychiatric drugs or drugs of abuse.

Reference	Specimen, Amount	Drugs Detected	LOD[1]	LLE[2]	SPE[3]	Ionisation Mode[4]	Drug Recovery
(Bogusz et al, 1998)	various tissues, 0.5-1.5 mL	opioids, cocaine, LSD, & metabolites; isocratic	0.1-100	--	Bond Elut C18	pos APCI	41-98%
(Cailleux et al, 1999)	urine/plasma/blood, 250 µL	opioids, cocaine & metabolites	5-10	chloroform/isopropanol	--	pos APESI-MS/MS	85-115%
(Decaestecker et al, 2000)	urine, 1 mL	opioids, cocaine, benzodiazepines; gradient	not stated	hexane/ethyl acetate	--	pos ESI-MS & MS/MS	not stated
(Muller et al, 2000)	hair, 50 mg	maprotiline, citalopram, pipamperone; gradient	<0.1	--	Chromabond Drug	pos ESI-CID & MS/MS	not stated
(Rittner et al, 2001)	serum, 1mL	70 psychoactive drugs; gradient	0.12-45	--	Isolute HCx & Bakerbond C18	pos ESI-CID	0-93%
(Tatsuno et al, 1996)	urine, 5 mL	stimulants & opioids; gradient	2-40	--	Sep-pak C18	thermospray	88-99%
(Weinmann et al, 2000a)	hair, 50 mg	6 neuroleptics; gradient	<0.05	--	Chromabond Drug	pos ESI-CID & MS/MS	53-92%

[1] LOD = limit of detection, expressed in ng/mL or ng/mg. [2] LLE = liquid-liquid extraction. [3] SPE = solid-phase extraction. [4] APCI = atmospheric pressure chemical ionisation, APESI = atmospheric pressure electrospray ionization, CID = collision-induced dissociation, MS/MS = tandem mass spectrometry.

Published LC-MS methods generally focus on the detection of only one drug or a small group of drugs, and has mainly involved analysis for drugs of abuse. A small number of these methods involve detection of more than one class of drug of abuse. However, three of these methods did not include benzodiazepines (Bogusz et al, 1998b; Cailleux et al, 1999; Tatsuno et al, 1996), and two did not include amphetamines (Cailleux et al, 1999; Decaestecker et al, 2000).

Only four papers have focused on LC/MS analysis of antidepressant drugs, although more have been published for detection of neuroleptics (Marquet, 2002). As with detection of drugs of abuse, however, most of these methods involve the detection of only one or a small number of drugs. Müller et al published a method for detection of a TCA, an SSRI, and a neuroleptic in one chromatographic run in hair (Muller et al, 2000a). The use of tandem mass spectrometry in this method provided enhanced sensitivity and selectivity, but most drugs in Table 1.9 were not included. Using essentially the same instrumental parameters, however, the same group extended the use of this method to a set of six neuroleptic drugs (Weinmann et al, 2000a). A robust method published by Rittner et al enabled the detection of 70 psychoactive drugs. Included were haloperidol, citalopram and sertraline, in addition to many drugs of abuse, although most opiates and some stimulants could not be detected with this method (Rittner et al, 2001). Regardless of the previously limited scope of application of the technique to toxicology, LC/MS shows great promise for combined screening and confirmation in one analysis.

1.9. Research plan

The principal aim of this dissertation was to investigate the toxicology of monoamine-active drugs. These drugs include antidepressants and antipsychotics that affect serotonin and dopamine systems as well as drugs of abuse that also affect serotonin and other monoamine receptor systems.

The dissertation includes sections on the development of appropriate methodology involving the use of LC-MS and related techniques for the detection of these psychiatric drugs in postmortem tissues, including brain. Modification of the established methods to enable detection of drugs of abuse in postmortem blood is also discussed. These methods were validated and used to study the tissue distribution of eight target psychiatric drugs and their metabolites, as well as the brain distribution of selected antipsychotics in schizophrenic subjects.

To more fully appreciate the significance of the concentrations of target psychiatric drugs in the postmortem setting, the effect of redistribution on postmortem drug concentrations was investigated. Lastly, police reports and coroners' findings were reviewed in selected cases for the involvement of target drugs in sudden unexpected death, by themselves or in combination. Observed trends were compared against the drugs' physicochemical properties to evaluate their relative toxicity. It is hoped that the findings from this research will contribute to a greater understanding of the toxicology of monoamine-active drugs.

CHAPTER 2 : GENERAL METHODOLOGY

2.1. Introduction

This chapter outlines details of case selection, collection of specimens, autopsy procedures, and storage protocols used for all specimens examined in this dissertation. Chromatographic and mass spectrometric assay development for the determination of psychiatric drugs is discussed in Chapter 3.

2.2. Case selection and collection of specimens

2.2.1. Case selection criteria

2.2.1.1. Tissue distribution and redistribution study

Fatalities in the State of Victoria were selected where the circumstances of death suggested the subject had taken the psychiatric drugs listed in Table 1.4. In each case, circumstances permitting, a separate set of specimens to those normally taken at autopsy was collected for research purposes. This included femoral blood, bile, urine, vitreous fluid, liver, and stomach contents. A section of the frontal cortex was also collected. To explore the possibility of postmortem redistribution, where possible, an additional blood samples was taken from the heart. Reports made available to the State Coroner's Office (such as police reports, autopsy findings,

witness statements and inquest findings) were used to determine the role of the drug in each death.

For quantitation purposes, blank blood for the preparation of calibration, control, and blanks were required. Expired blank blood was obtained with permission from the Red Cross Blood Bank, or taken from staff members at the Victorian Institute of Forensic Medicine (VIFM) by a qualified medical practitioner. For quantitation of other tissues, blank specimens were selected after routine toxicology screens returned negative results.

2.2.1.2. Study of postmortem antipsychotic drug distribution in schizophrenic brains

Another aspect of this dissertation involved investigating postmortem antipsychotic drug distribution in schizophrenic brains. To accomplish this, specimens from a variety of brain regions were taken at autopsy from 22 subjects with a provisional diagnosis of schizophrenia. Regions sampled included caudate-putamen, grey and white matter from the frontal cortex, occipital cortex, and cerebellum.

The provisional diagnosis of schizophrenia was made based on police reports of death to the Coroner. This diagnosis was, however, confirmed by a senior psychologist and senior psychiatrist after an extensive case history review following collaboration with the Mental Health

Research Institute (MHRI, Dr. Brian Dean) (Hill et al, 1996). Brain specimens from 11 subjects with no history of psychiatric illness, and matched for sex and of a similar age to the schizophrenic subjects, were used as controls.

2.2.1.3. Coronial cases for drug of abuse method development

Femoral blood and urine specimens known to contain one or more drugs of abuse based on positive results obtained in routine GC/MS and HPLC drug screening methods were selected for re-analysis. Results obtained in this research were compared to those obtained using conventional methods in the routine toxicology laboratory.

2.2.2.   Ethics approval process

Approval for the use of human blood and tissues was granted by the VIFM Ethics Committee, according to established guidelines. In each case in which circumstances suggested suitability for inclusion, the next-of kin was provided a written summary of the objectives and requirements of this research (Appendix A). Only those cases in which consent was given by the senior next of kin were used. This process was conducted by the Donor Tissue Bank of Victoria (DTBV).

2.3. Specimen collection and storage protocols

All blank blood from the Red Cross Blood Bank contained the red cell preservative ADSOL®, the components of which are shown in Table 2.1. Upon receipt at the laboratory, the blood was decanted into 10 mL polypropylene tubes containing sodium fluoride/potassium oxalate preservative such that the final concentration was 1 % (w/v) (Biolab, Australia).

All blood taken from VIFM staff members was collected into polypropylene tubes containing the same sodium fluoride/potassium oxalate mixture. Prior to use as blank blood, these specimens were analysed using routine drug screening methods adopted by the laboratory. These included GC/MS screening of basic/neutral drugs, HPLC and Enzyme Multiplied Immunoassay Technique (EMIT) for drugs of abuse (Asselin and Leslie, 1994; Drummer et al, 1993a; Drummer et al, 1994).

Table 2.1. Components in 100 mL ADSOL® Red Cell Preservative Solution.

Component	Amount (mg)
Dextrose (monohydrate)	2200
Sodium Chloride	900
Mannitol	750
Adenine	27

Mortuary technicians collected all postmortem specimens. Blood specimens were collected in 10 mL polypropylene tubes, containing 1 % (w/v) sodium fluoride/potassium oxalate preservative. Specimens, once acquired, were stored at -20 °C. Schizophrenic and control brain specimens (2.5 g) were collected at autopsy and were rapidly frozen to -70 °C. Specimens were stored at this temperature until required. Other specimens were collected as shown in Table 2.2.

2.4. Calculation of postmortem interval

Where a subject's death had been witnessed, the postmortem interval (PMI) was defined as the time of death to autopsy. In cases where the body was found and no witnesses to the death existed, the PMI was estimated at half way between the donor being found dead and being last seen alive. In all cases, the cadavers were refrigerated within 5 hours of being found.

2.5. Solid tissue homogenisation

Prior to extraction and analysis for drugs, solid organs (liver and brain) were homogenised. A portion of brain or liver was cut and placed in a weighing tray (~2.5 g or ~10 g, respectively), and the exact weight recorded. The specimen was finely diced, and an equal volume of deionised water was added. The homogenisation process differed at this point for the two specimen types.

Table 2.2. Tissues obtained at autopsy in suicides and known or suspected drug overdoses.

Type	Tissue	Site	Weight/Volume (max)	Specimen Container
Tissue Distribution & Redistribution	Blood	Femoral Vein	10 mL	polypropylene tube containing 1% sodium fluoride/potassium oxalate
	Blood	Heart	10 mL	polypropylene tube containing 1% sodium fluoride/potassium oxalate
	Bile	Gall bladder	10 mL	polypropylene tube
	Brain	Frontal Cortex	10 g	plastic pot
	Gastric Contents	Stomach	500 g (max)	plastic pot
	Liver	Liver	50 g	plastic pot
	Urine	Bladder	10 mL	polypropylene tube
	Vitreous fluid	Vitreous body	10 mL	polypropylene tube

Type	Tissue	Site	Weight/Volume (max)	Specimen Container
Antipsychotic Drug Distribution in Schizophrenic Brain	Brain	Caudate-putamen	2.5 g	plastic bag
	Brain	Frontal Cortex (grey)	2.5 g	plastic bag
	Brain	Frontal Cortex (white)	2.5 g	plastic bag
	Brain	Occipital Cortex	2.5 g	plastic bag
	Brain	Cerebellum	2.5 g	plastic bag
Drug of Abuse Method Development	Blood	Femoral vein	10 mL	polypropylene tube containing 1% sodium fluoride/potassium oxalate

2.5.1. Brain

The brain/water mixture was transferred to a 15 mL plastic pot and homogenized for approximately 5 minutes using an ultrasonic probe (Ultraturbax T25, IRA Labortechnik, Bremen, Germany) until the mixture was smooth. These tissue homogenates were then stored at -20 °C until use. For quantitation, all brain concentrations were multiplied by a correction factor (CF) to provide a measure of the amount of tissue in a homogenate from a measured volume. The formula for this calculation as used by Gerastomoulos (Gerastomoulos, 1997) follows:

$CF = (M_t$- theoretical weight in mg/kg)$/M_{tr}$-real weight

where T_w = wet tissue weight in g

V_{water} = volume of H_2O added in mL

M_t = mass of T_w + V_{water} (theoretical weight of the tissue + H_2O)

M_{tr} = mass of T_1 + V_{water} (real weight of the tissue + H_2O)

This formula was derived from a series of experiments and allowed conversion of concentrations in mg/L to mg/kg when only a portion of the homogenate was used. The brain concentration of drug (expressed in mg/L) was multiplied by the CF (determined to be 1.4) to obtain a concentration in mg/kg.

2.5.2. Liver

The liver/water mixture was transferred to a plastic homogenising bag and placed in a stomacher for 5 min (Lab-Blender 80, Seward Medical, London, UK). The mixture was removed from the bag, placed in a 40 mL plastic pot, and homogenised for approximately 5 minutes using an ultrasonic probe (Ultraturbax T25, IRA Labortechnik, Bremen, Germany). The pH was adjusted to 10.0 with 10 M NaOH. Subtilisin (10 mg, Sigma Aldrich Pty Ltd (Castle Hill, NSW, Australia) was added and the homogenate incubated at 55 °C for 1 h. Following incubation, the pH was readjusted to 7.0 using 1 M HCl, and the specimen stored at -20 °C. As for brain, all liver concentrations were multiplied by a CF (1.5 for liver) to obtain results in mg/kg.

2.6. Routine VIFM toxicology procedures

The toxicology section at VIFM retains all autopsy specimens, which are routinely subjected to a wide variety of screening analyses. Urine specimens are tested using an EMIT screen for drugs of abuse (amphetamines, cocaine metabolites, benzodiazepines, cannabinoids, and opiates). A basic/neutral drug screen is also conducted using capillary gas chromatography with dual nitrogen-phosphorous detection (GC/NPD) and mass spectrometry (GC/MS), a GC assay for blood-alcohol concentration, and an HPLC screen (Drummer et al, 1993a; Drummer et al, 1994; Franke and al, 1988; Ramsay and Flanagan, 1982). Depending on results from the various screens, confirmation and quantitation of drugs is then performed. Generally, whole blood specimens are used, although other specimens are sometimes also analysed. For these analyses, this laboratory typically employs HPLC and GC/MS techniques which have all been validated

according to established procedures. Quality control specimens are routinely analysed to monitor

each assay's performance. The toxicology laboratory is accredited by the National Australian

Testing Association (NATA) and the American Society of Crime Laboratory Directors-

Laboratory Accreditation Board (ASCLD-LAB).

CHAPTER 3 : PSYCHIATRIC DRUG ASSAY

DEVELOPMENT

3.1. Introduction

The detection of psychiatric drugs as a group is reviewed in Section 1.8. A number of papers have been published on the detection of atypical antidepressants using either gas chromatography (GC) (Crifasi et al, 1997; Lefebvre et al, 1999; Long et al, 1997) or HPLC (Alvarez et al, 1998; Balant-Gorgia et al, 1999; Begg et al, 1999; Laroudie et al, 1999; Vatassery et al, 1997). None of the HPLC methods used mobile phases with pH's above 8.0. Moreover, these papers all involved detection of only one drug and its major metabolite, or a small group of drugs and metabolites. While practical in therapeutic drug monitoring, methods focusing on a small number of drugs are not as useful in a forensic toxicology laboratory when there is a need to screen and confirm a larger number of drugs. Of those methods that target multiple drugs (see Section 1.8), none have been used to analyse all of the psychiatric drugs listed in Table 1.4.

The drugs that were selected for this dissertation represent the variety of psychiatric drugs typically found in Coroners' cases in Australia. At the time of this dissertation, there was no published method capable of measuring all of the drugs listed in Table 1.4 in one chromatographic system, using LC-MS or any other analysis technique. This chapter outlines the validation of the LC-MS method used to analyse various biological specimens containing these drugs.

3.2. Materials and methods

3.2.1. Materials

9-OH-risperidone was obtained from Janssen-Cilag Pty Ltd (North Ryde, Australia), and trazodone from Sigma Aldrich Pty Ltd (Castle Hill, Australia). All other drug standards were obtained from the Division of Analytical Laboratories of the New South Wales Health Department (Lidcombe, Australia). Reagents were HPLC grade or better and were obtained from Sigma Aldrich Pty Ltd.

For methods development, 1 mg/mL stock solutions of standards were prepared in methanol monthly and stored at -20 °C until use. To enable a comparison of methods, 5 mg/L working standards were prepared fresh daily with deionized water.

3.2.2. Specimen preparation

The extraction technique used in this dissertation for the determination of psychiatric drugs in postmortem blood and other tissues was based on a procedure routinely used at the VIFM (McIntyre et al, 1993a) and which was modified for use with LC-MS. To a 1 mL specimen of blood or other tissue collected at autopsy, deionized water and 0.2 M sodium carbonate buffer (1 mL each), and 100 ng internal standard (trazodone), were mixed and vortexed. Butyl chloride (6 mL) was added and the samples rotated for 20 min, and then centrifuged at 3000 rpm for 5 min.

The organic fraction was transferred to another tube and the aqueous portion was back-extracted with 0.2 % phosphoric acid (100 µL).

The excess solvent was dried and the aqueous layer basified with 100 µL each of concentrated (28%) ammonia solution (Sigma Aldrich Pty Ltd) and saturated ammonium carbonate, then re-extracted with chloroform (100 µL). The chloroform fraction was evaporated to dryness and reconstituted in 100 µL mobile phase. 30 µL of this solution was injected into the LC-MS for analysis.

3.2.3. Instrumental conditions

3.2.3.1. HPLC

HPLC analysis was performed on a 1100 Series HPLC (Agilent Technologies, Forest Hill, Australia) with a G1315A photodiode array detector (DAD) monitoring at wavelengths of 220 and 254 nm. The analytical system was linked to an IBM-compatible PC with ChemStation 4900 Series software. Chromatographic separation was achieved using a Zorbax Extend-C_{18} column from Agilent (2.1 x 150 mm, 5 µm particle size). The column was operated at 21 °C pumping at 0.25 mL/min for 40 min. To enable the use of this method in LC-MS analysis, volatile organic buffers were used. In developing the method, 0.05M solutions of ammonia (pK_a=9.2), glycine (pK_a=9.8), and triethylamine (pK_a=10.7) were tested for use as buffers, and tetrahydrofuran (THF) and acetonitrile (AcN) were used as modifying agents. Mobile phases titrated to pH's ranging from 8.5 to 10.5 were tested. 12M formic acid was used to titrate the buffer to the

desired pH prior to mixing with methanol. The optimum mobile phase used in blood and tissue

analyses consisted of 0.05M ammonia/methanol/THF (32.5:67.0:0.5) at pH 10.0.

3.2.3.2. LC-MS

LC-MS analysis was performed on a 1100 Series HPLC (Agilent Technologies, Forest Hill,

Australia) configured with a G1946A mass selective detector (MSD) operating in Selected Ion

Monitoring (SIM) mode. Table 3.1 shows the ions monitored for each drug.

Table 3.1. Monitored ions of serotonergic drugs in mass spectral analysis, in elution order[1].

Compound	Ions Monitored[2]		
	Quant ion	Qualifier 1	Qualifier 2
O-desmethylvenlafaxine	264 (100)	265 (18.5)	246 (19)
9-OH-risperidone	427 (100)	428 (26.2)	429 (4)
Risperidone	411 (100)	412 (26)	414 (3.8)
Trazodone	372 (100)	373 (23)	374 (34.5)
Fluvoxamine	319 (100)	320 (17.5)	321 (1.75)
Venlafaxine	278 (100)	279 (20)	260 (13.2)
Norfluoxetine	296 (100)	366 (29)	134 (26)
Paroxetine	330 (100)	331 (1.8)	332 (0.2)
Fluoxetine	310 (100)	311 (17)	148 (21.5)
N-desmethylsertraline	275 (100)	277 (66)	159 (25)
Nefazodone	470 (100)	471 (29.5)	472 (36.5)
Sertraline	275 (100)	277 (65)	159 (7)

[1] The m/z of the base peak for each drug is shown in bold.

[2] relative ion abundances (expressed in %) shown in parentheses.

The column, operating temperature and pressure, and mobile phase were as discussed previously in section 3.2.3.1 for HPLC. Mass spectral detection was achieved using atmospheric pressure electrospray ionisation in positive mode, with a fragmentor voltage setting of 80 V. The nebulizer pressure was set at 30 psig and the capillary voltage at 3500 V. The drying gas (nitrogen) flow rate was set at 10 L/min at a temperature of 325 °C.

3.2.4. Comparison of analytical results

Analytical results were compared on the basis of retention factors and resolution of seven pairs of closely eluting compounds (see Table 3.2.). Resolution, R_s, and retention factors, k, were calculated according to Miller (Miller, 1987), where R_s is a function of retention time and peak width of the drugs of interest, and k is a function solely of retention time. Values for each were calculated according to the following formulae:

$$R_s = (t_{R,2} - t_{R,1})/(w_1 + w_2)$$
$$k = t_R'/t_M$$
$$t_R' = t_R - t_M$$

Where $t_{R,1}$ = retention time of the earlier eluting compound

$t_{R,2}$ = retention time of the later eluting compound

w_1 = baseline peak width of the earlier eluting compound

w_2 = baseline peak width of the later eluting compound

t_M = retention time of a non-retained compound

t_R' = net retention time

Table 3.2. Drug pairs used for resolution calculations.

Peak Pair	Compound Names
1	O-desmethylvenlafaxine & 9-OH-risperidone
2	9-OH-risperidone & Risperidone
3	Fluvoxamine & Venlafaxine
4	Fluvoxamine & Norfluoxetine
5	Venlafaxine & Norfluoxetine
6	Norfluoxetine & Paroxetine
7	N-desmethylsertraline & Nefazodone

Analytical results were compared on the basis of drug recovery, intra- and inter-assay precision (measured as coefficients of variation, or CV's), and accuracy. Recovery was calculated by comparing the peak area of an extracted specimen spiked with a known drug concentration to that of unextracted standards of known concentration, and expressed as a percentage. CV's were calculated using the standard deviation (σ) and mean (x) of replicate analyses of specimens spiked with known concentrations using the formula $CV = (\sigma/x) \cdot 100$. Finally, accuracy was calculated by dividing the measured concentration by the calculated concentration and multiplying the result by 100. The optimum mobile phase was used to analyse replicate 1 mg/L standards of the same mixture of drugs spiked in drug-free blood. The limit of quantitation for mass spectral detection was defined as the level which consistently gave CV's < 20 % in SIM mode.

3.3. Results

3.3.1. Effect of buffer choice on resolution

Figure 3.1 shows the relationship between buffer choice and resolution of the seven drug pairs listed in Table 3.2. Mobile phases buffered with either ammonia (pK_a=9.2) or glycine (pK_a=9.8) separated the drug mixture substantially better at pH 10.0 than the mobile phase buffered with triethylamine, which has a pK_a of 10.7. Using glycine as the buffer, peak pairs were best resolved using a mobile phase composition of 0.05 M glycine/methanol/THF (32.5:67.0:0.5, v/v). When triethylamine was used as the buffering agent, the best resolution was achieved using a mobile phase comprised of 0.05 M triethylamine/methanol/THF (32.5:64.5:3.0, v/v).

3.3.2. Effect of modifying agents on resolution

At pH 10.0, ammonia best resolved the drug pairs listed in Table 3.2 with a mobile phase of 0.05 M ammonia/methanol (32.5:67.5, v/v). THF and acetonitrile were added as modifying agents to reduce runtime. It was found that the runtime could be shortened while still providing resolution of 1.5 or better for all drug pairs by adding 0.5 % THF. The effect of both THF and AcN on resolution of each peak pair compared to mobile phase containing neither modifier is shown in Figure 3.2.
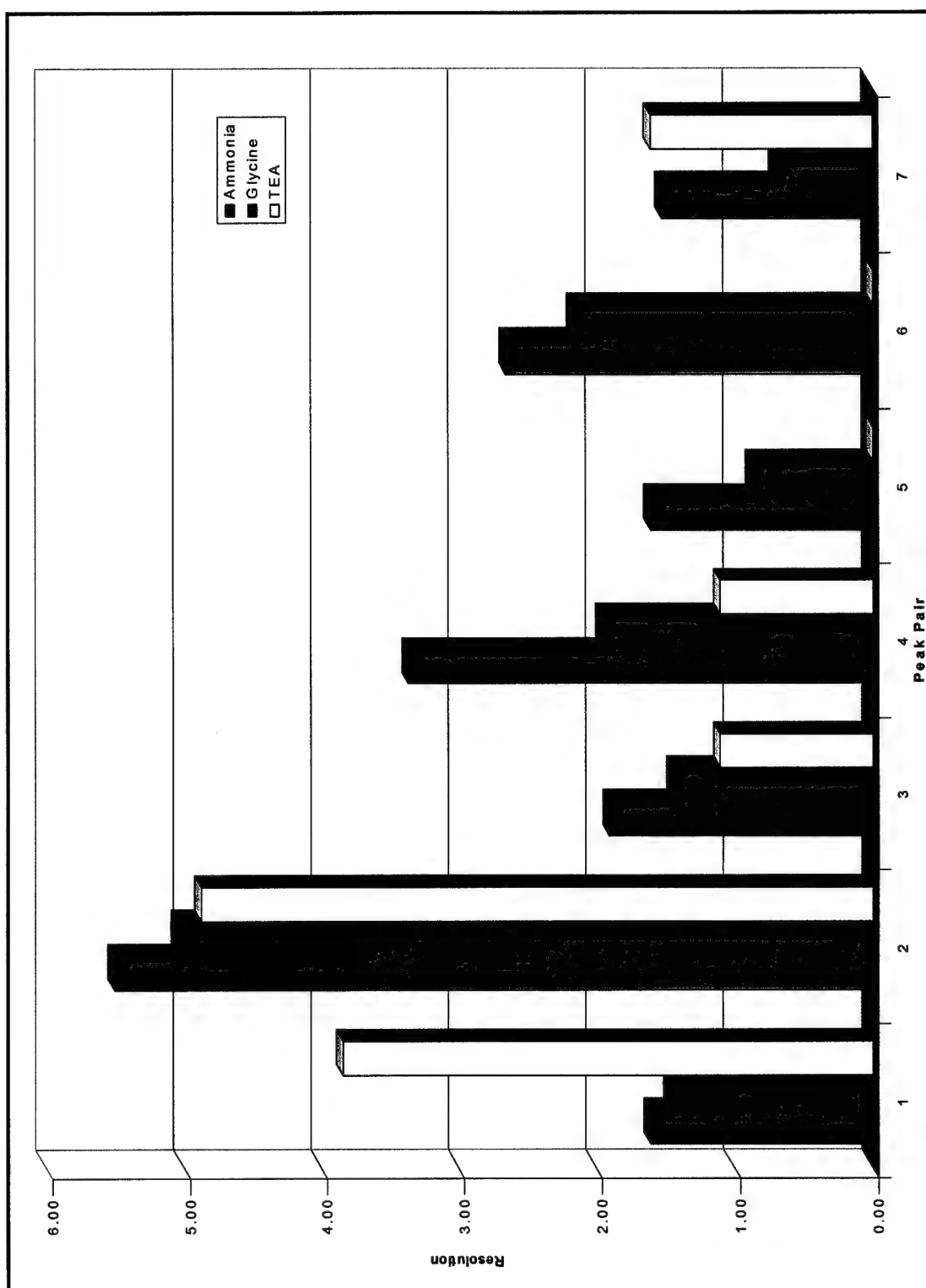
Figure 3.1: The relationship between buffer choice and resolution of selected drug pairs defined in Table 3.2 (32.5:67.0:0.5 (v/v) buffer/methanol/THF using DAD).
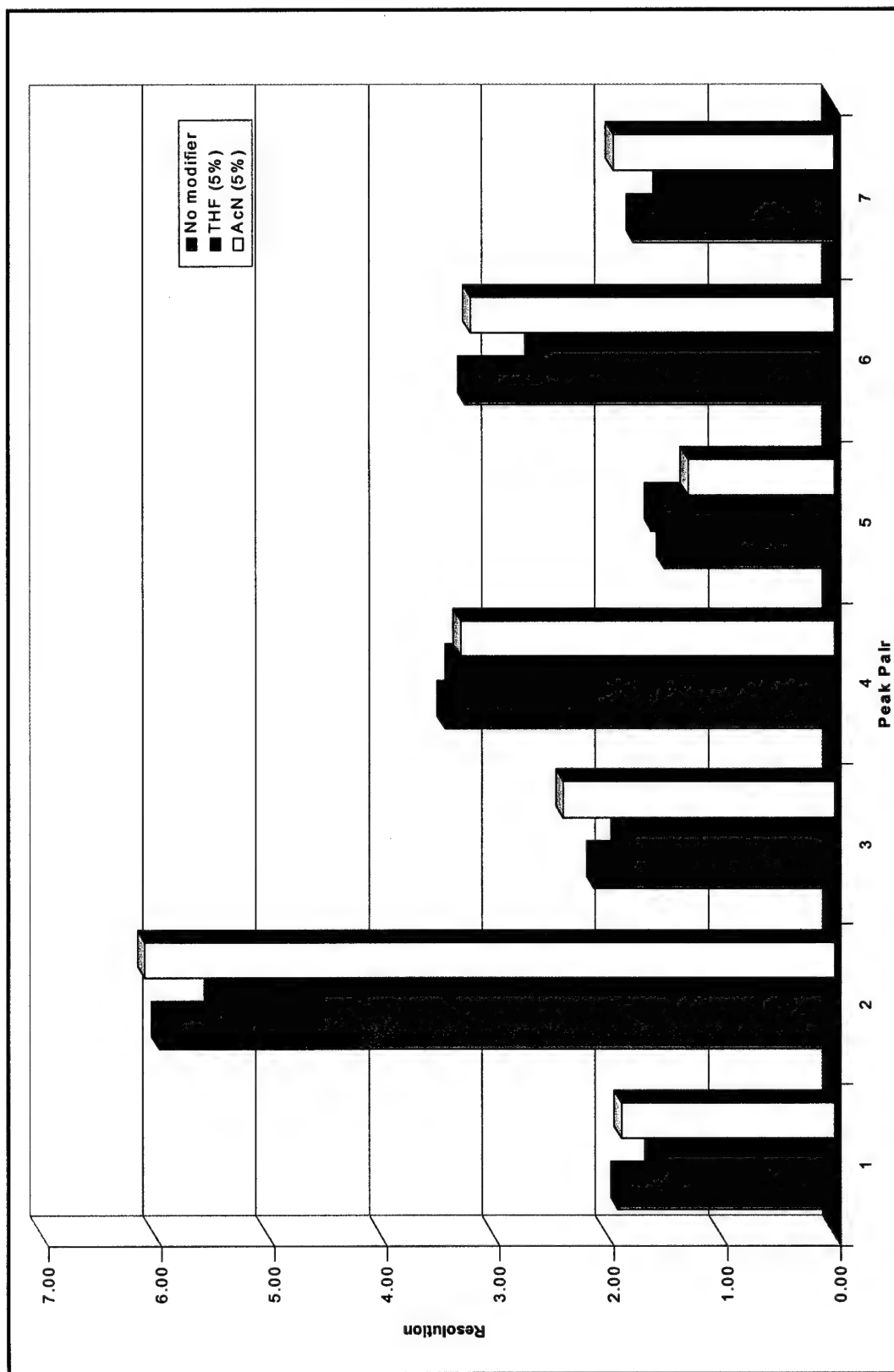
Figure 3.2. The effect of modifying agent selection on resolution of selected drug pairs defined in Table 3.2 -- 32.5:67.0:0.5 (v/v) ammonia/methanol/modifying agent (67.5% methanol used when no modifying agent employed).

3.3.3. Effect of pH on resolution and mass spectral peak height

Chromatography performed at pH<10.0 reduced runtime but caused many compounds to coelute, resulting in decreased detection selectivity when using DAD detection. (Figure 3.3). At pH > 10.0, band broadening diminished resolution. Regardless of buffer choice, all seven peak pairs were resolved best using mobile phases titrated to 10.0.

Mobile phase pH also had a consistent effect on base peak abundance in the mass spectra of the compounds studied (Figure 3.4). For each drug, base peak abundance increased with increasing pH. A mobile phase pH of 10.0 was chosen for assays carried for this dissertation in order to optimise resolution as well as mass spectral base peak height.

A representative set of chromatograms from LC-MS analysis using the optimised method is included in Figure 3.5. Even though all drugs were not completely resolved using this method of detection, quantitation could still be carried out by using selected ion monitoring (SIM) and examining the relevant individual selected ions.
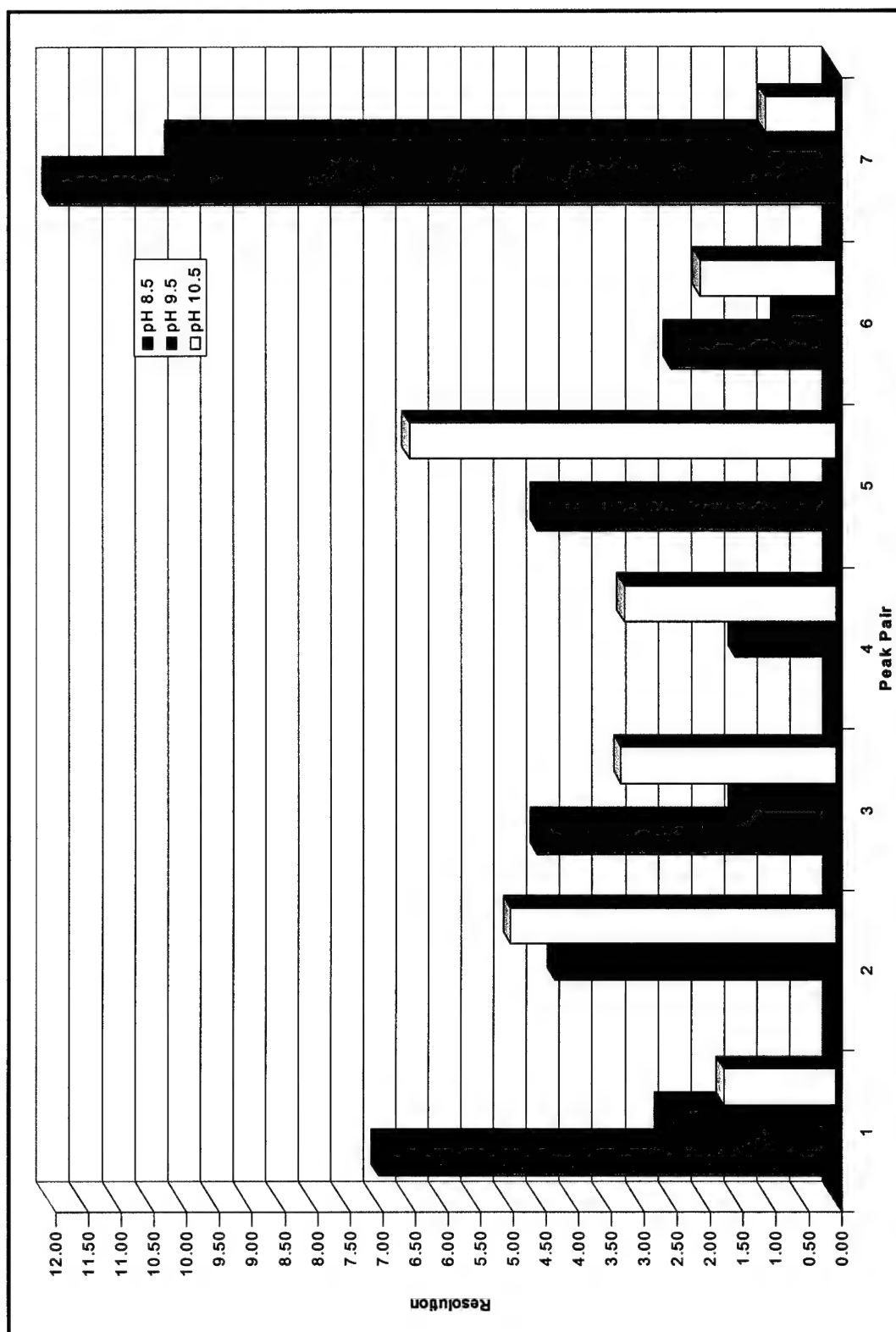
Figure 3.3: The effect of pH variation on chromatographic separation on peak pairs listed in Table 3.2 — 32.5:67.0:0.5 (v/v) ammonia/methanol/THF.
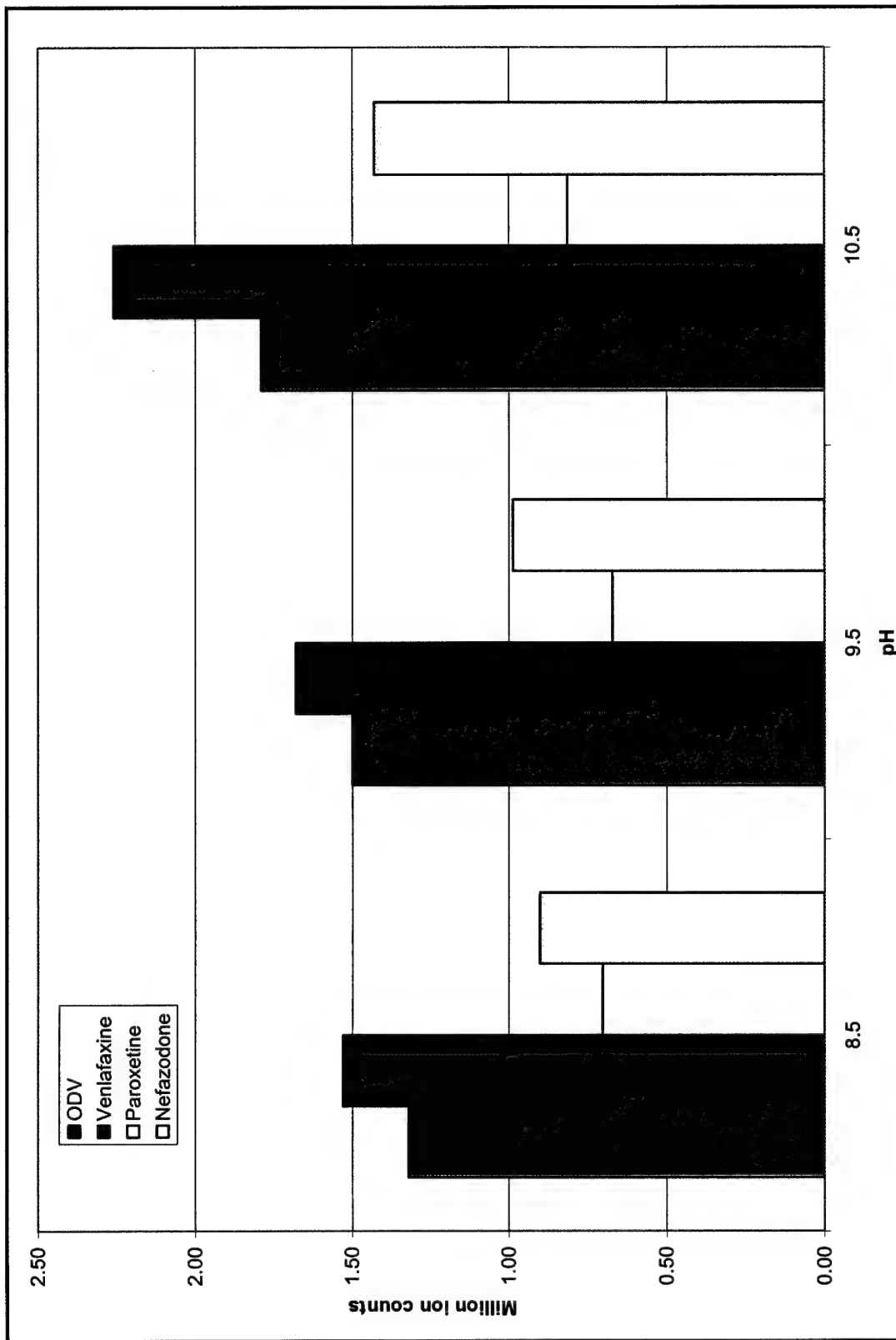
Figure 3.4. Effect of pH on mass spectral base peak abundance for selected psychiatric drugs (32.5:67.0:0.5 (v/v) ammonia/methanol/ THF).
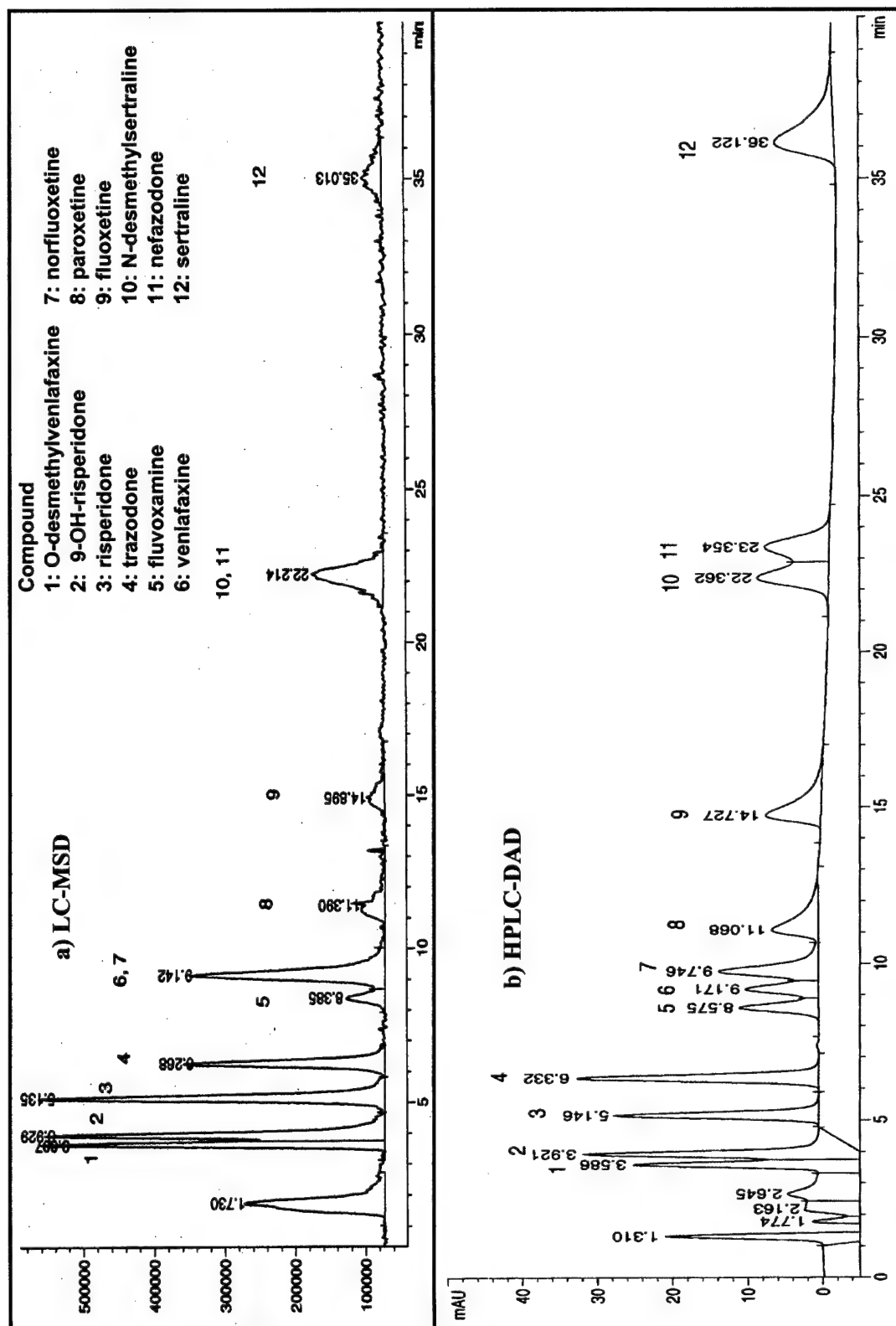
Figure 3.5: Results obtained using 32.5:67.0:0.5 (v/v) ammonia/methanol/THF with a 2.1 x 150 mm Zorbax Extend C_{18} column using a) LC-MSD and b) HPLC-DAD to analyze a neat standard (30 μL injected).

Tables 3.3-3.8 include correlation coefficients, recoveries, accuracy and precision data for selected serotonergic drugs and metabolites at concentrations of 0.075 mg/L (low) and 1.0 mg/L (high) in various tissues using the optimised method. Within- and between-day coefficients of variation were all below 10%. The limit of quantitation was 50 ng/mL for 9-OH-risperidone, paroxetine, N-desmethylsertraline, and sertraline, and 10 ng/mL for the remaining drugs. Calibration curves passed through the origin and were linear to at least 5000 ng/mL with r^2 values of 0.99 or better. Recoveries were 78-104 % for all drugs. Chromatograms of tissue extracts showed the presence of endogenous peaks with retention times ranging from 0 to 2.6 minutes. Additionally, peaks at 6.7 min (3.7 min if a 2.1 x 50 mm column was used) and 16.6 min appeared in most cases (Figure 3.6). These peaks were higher in liver and bile extracts and in highly decomposed specimens. However, none of these peaks appeared in the extracted ion chromatograms for any drugs and thus they did not interfere with quantitation. Using a mobile phase comprised of 32.5:67.0:0.5 (v/v) 0.05M ammonia/methanol/THF, the relative standard deviation (RSD) of retention times ranged from 0.6 % for paroxetine to 6.0 % for fluvoxamine, with an average RSD of 2.5 % (see Table 3.9).

Table 3.3. Typical recoveries, linearity, precision, and accuracy data for 13 psychiatric drugs in blood spiked with 0.075 mg/L (low) and 1.0 mg/L (high) drug standards.

Compound	Regression Equation	r^2	intra-assay CV (N=5)		inter-assay CV (N=5)		Acc (%)	Rec (%)
			low	high	low	high		
O-desmethylvenlafaxine	y = 0.33x + 0.04	0.996	11.3	4.41	11.3	7.64	100	88.0
9-OH-risperidone	y = 0.13x - 0.003	0.998	6.50	3.64	13.3	9.20	111	78.0
Risperidone	y = 0.15x - 0.01	0.996	3.23	4.24	4.40	3.97	102	116
Citalopram	y = 0.14x - 0.002	0.999	5.00	4.44	10.2	7.19	118	100
Fluvoxamine	y = 0.43x-0.004	1.00	2.38	2.29	11.8	6.74	97.0	103
Norfluoxetine	y = 0.12x - 0.002	0.999	4.03	4.68	6.81	6.63	108	101
Venlafaxine	y = 0.68x + 0.14	0.993	3.36	2.77	9.03	4.40	115	98.4
Paroxetine	y = 0.54x + 0.05	0.991	3.03	3.19	6.54	5.30	105	101
Fluoxetine	y = 0.14x - 0.004	1.00	4.33	3.50	5.77	5.51	106	99.2
N-desmethylsertraline	y = 0.13x - 0.005	0.999	5.56	2.96	7.13	6.77	115	101
Nefazodone	y = 0.25x + 0.18	0.991	4.11	2.35	8.71	4.55	102	101
Sertraline	y = 0.14x - 0.006	0.999	3.15	3.41	6.87	5.84	107	101

Table 3.4. Typical recoveries, linearity, precision, and accuracy data for 13 psychiatric drugs in bile spiked with 0.075 mg/L (low) and 1.0 mg/L (high) drug standards.

Compound	Regression Equation	r^2	intra-assay CV (N=5)		inter-assay CV (N=5)		Acc (%)[1]	Rec (%)[1]
			low	high	low	high		
O-desmethylvenlafaxine	y = 0.25x - 0.01	1.00	5.14	3.27	8.36	5.23	107	99.1
9-OH-risperidone	y = 9.18x - 0.02	0.997	5.27	5.50	8.18	8.93	101	103
Risperidone	y = 0.14x - 0.004	0.998	3.87	4.76	6.07	6.61	108	96.4
Citalopram	y = 0.23x - 0.05	0.991	5.76	8.58	5.58	3.22	107	104
Fluvoxamine	y = 0.14x - 0.005	0.999	5.08	5.62	4.78	4.07	102	103
Norfluoxetine	y = 0.16x + 0.003	0.999	5.17	2.69	8.85	7.39	101	101
Venlafaxine	y = 0.15x + 0.002	0.998	6.60	3.26	7.78	6.76	101	98.4
Paroxetine	y = 0.15x - 0.002	1.00	5.79	4.91	6.96	5.13	119	101
Fluoxetine	y = 0.14x + 0.004	0.997	4.75	4.59	5.68	4.41	109	99.2
N-desmethylsertraline	y = 0.14x - 0.005	0.998	7.05	3.95	5.67	6.09	206	97.1
Nefazodone	y = 0.14x - 0.004	0.999	4.26	1.68	7.65	8.10	102	99.3
Sertraline	y = 0.19x - 0.01	0.996	8.24	4.27	8.34	4.17	105	101

[1] Calculated using 1.0 mg/L standards.

Table 3.5. Typical recoveries, linearity, precision, and accuracy data for 13 psychiatric drugs in frontal cortex spiked with 0.075 mg/L (low) and 1.0 mg/L (high) drug standards.

Compound	Regression Equation	r^2	intra-assay CV (N=5) low	high	inter-assay CV (N=5) low	high	Acc (%)[1]	Rec (%)[1]
O-desmethylvenlafaxine	y = 0.19x - 0.03	0.997	3.62	3.35	8.13	6.76	97.3	97.8
9-OH-risperidone	y = 0.13x + 0.002	0.999	5.26	2.70	8.72	6.98	102	102
Risperidone	y = 0.13x + 0.002	0.999	6.01	2.18	8.69	6.18	100	99.2
Citalopram	y = 0.15x - 0.003	0.999	6.84	5.30	6.10	5.34	108	102
Fluvoxamine	y = 0.11x - 0.002	1.00	4.75	4.56	8.96	6.97	107	100
Norfluoxetine	y = 0.12x - 0.02	0.997	6.15	3.86	9.62	9.12	109	96.6
Venlafaxine	y = 0.12x - 0.01	0.997	3.96	2.56	7.25	6.97	111	100
Paroxetine	y = 0.12x- 0.002	0.999	3.46	2.44	8.82	6.13	104	104
Fluoxetine	y = 0.11x + 0.003	1.00	4.38	2.36	5.68	5.41	98.3	100
N-desmethylsertraline	y = 0.12x - 0.008	0.997	4.20	2.06	5.67	6.09	99.6	99.9
Nefazodone	y = 0.15x - 0.01	0.998	4.76	2.93	7.65	8.10	106	101
Sertraline	y = 0.10 x + 0.004	1.00	4.78	3.07	8.34	5.17	99.7	101

1 Calculated using 1.0 mg/L standards.

Table 3.6. Typical recoveries, linearity, precision, and accuracy data for 13 psychiatric drugs in liver spiked with 0.075 mg/L (low) and 1.0 mg/L (high) drug standards.

Compound	Regression Equation	r^2	intra-assay CV (N=5)		inter-assay CV (N=5)		Acc (%)[1]	Rec (%)[1]
			low	high	low	high		
O-desmethylvenlafaxine	y = 0.21x - 0.006	0.999	4.32	2.70	8.10	6.96	99.0	102
9-OH-risperidone	y = 0.16x - 0.03	0.991	7.98	5.16	8.09	5.57	107	95.1
Risperidone	y = 0.29x - 0.04	0.995	6.28	5.41	6.91	6.24	99.2	98.6
Citalopram	y = 0.15x - 0.01	0.996	8.45	3.98	8.27	8.19	101	100
Fluvoxamine	y = 0.12x - 0.002	0.997	6.03	2.85	8.89	5.64	106	94.8
Norfluoxetine	y = 0.15x - 0.008	0.999	4.61	2.85	8.13	8.15	103	101
Venlafaxine	y = 0.12x - 0.005	0.997	6.36	6.11	10.1	8.12	105	98.7
Paroxetine	y = 0.12x -0.002	0.999	6.38	2.36	5.42	4.73	99.6	101
Fluoxetine	y = 0.22x - 0.02	0.997	3.18	2.92	8.37	5.23	102	100
N-desmethylsertraline	y = 0.22x - 0.02	0.998	2.89	2.35	8.26	4.79	101	96.7
Nefazodone	y = 0.14x + 0.009	0.995	4.14	4.13	6.49	6.00	108	102
Sertraline	y = 0.18x + 0.002	1.00	5.47	2.66	5.82	5.26	101	96.7

[1] Calculated using 1.0 mg/L standards.

Table 3.7. Typical recoveries, linearity, precision, and accuracy data for 13 psychiatric drugs in urine spiked with 0.075 mg/L (low) and 1.0 mg/L (high) drug standards.

Compound	Regression Equation	r^2	intra-assay CV (N=5)		inter-assay CV (N=5)		Acc (%)[1]	Rec (%)[1]
			low	high	low	high		
O-desmethylvenlafaxine	y = 0.16x - 0.007	0.998	6.46	5.46	8.69	6.69	107	100
9-OH-risperidone	y = 0.22x + 0.009	0.999	5.28	4.99	6.50	4.65	100	97.9
Risperidone	y = 0.14x + 0.006	0.996	8.31	3.95	6.12	5.24	101	98.7
Citalopram	y = 0.15x + 0.008	0.995	4.06	2.63	6.20	6.83	99.7	98.5
Fluvoxamine	y = 0.13x - 0.003	0.999	7.01	6.83	6.28	4.32	109	97.8
Norfluoxetine	y = 0.15x - 0.003	0.998	7.00	3.50	7.00	5.34	105	94.3
Venlafaxine	y = 0.17x - 0.005	0.998	6.84	5.01	6.77	5.80	111	102
Paroxetine	y = 0.15x - 0.007	0.998	9.62	4.17	6.00	5.72	105	101
Fluoxetine	y = 0.21x - 0.02	0.994	8.82	3.47	8.42	5.91	103	101
N-desmethylsertraline	y = 0.20x - 0.005	1.00	8.79	3.55	9.90	5.91	95.7	99.0
Nefazodone	y = 0.24x -0.004	0.999	8.98	3.00	10.1	6.87	98.3	102
Sertraline	y = 0.14x - 0.001	0.999	7.54	3.41	8.79	8.55	101	99.7

[1] Calculated using 1.0 mg/L standards.

Table 3.8. Typical recoveries, linearity, precision, and accuracy data for 13 psychiatric drugs in vitreous fluid spiked with 0.075 mg/L (low) and 1.0 mg/L (high) drug standards.

| Compound	Regression Equation	r^2	intra-assay CV (N=5)		inter-assay CV (N=5)		Acc (%)[1]	Rec (%)[1]
			low	high	low	high		
O-desmethylvenlafaxine	y = 0.21x + 0.008	0.993	6.58	4.13	8.61	6.65	96.0	96.8
9-OH-risperidone	y = 0.71x + 0.008	0.998	6.39	4.48	6.38	5.87	98.5	102
Risperidone	y = 0.74x + 0.01	0.992	3.04	4.18	5.44	5.40	106	102
Citalopram	y = 0.19x + 0.003	0.990	7.44	4.44	6.70	6.45	101	97.0
Fluvoxamine	y = 0.15x + 0.002	0.998	7.01	2.39	8.92	6.77	100	103
Norfluoxetine	y = 0.25x + 0.005	0.991	4.36	3.20	5.50	6.64	102	101
Venlafaxine	y = 0.72x + 0.005	0.997	8.82	4.34	8.02	8.29	104	101
Paroxetine	y = 0.37x - 0.004	0.996	10.4	3.22	6.00	5.90	106	101
Fluoxetine	y = 0.16x + 0.005	0.990	3.50	4.39	7.00	5.28	105	104
N-desmethylsertraline	y = 0.14x + 0.007	0.994	5.80	3.78	9.77	8.46	108	102
Nefazodone	y = 0.15x + 0.002	0.995	5.24	4.03	8.39	6.29	108	98.9
Sertraline	y = 0.53x + 0.001	0.991	8.84	3.57	10.7	6.36	108	104

[1] Calculated using 1.0 mg/L standards.
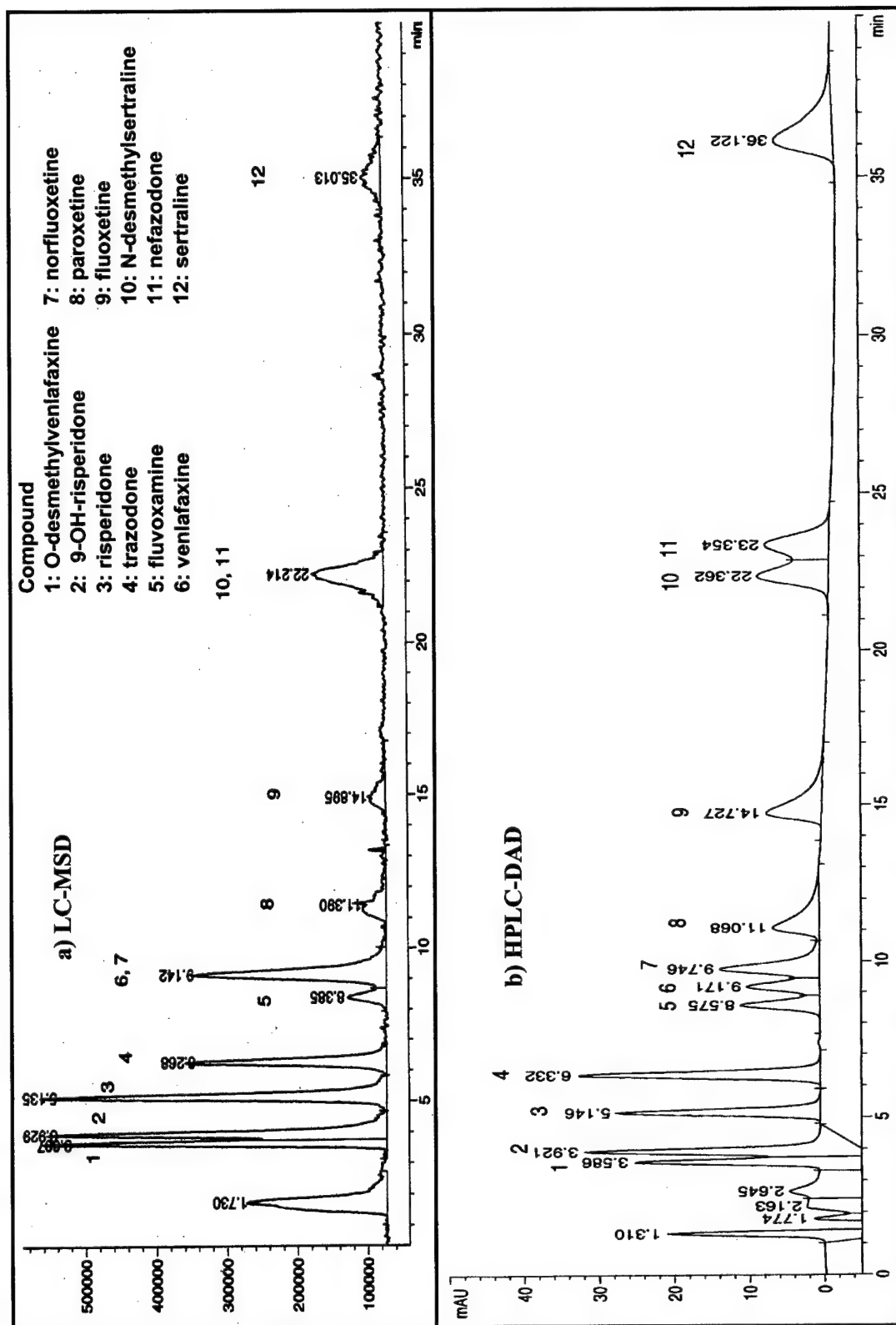
Figure 3.5: Results obtained using 32.5:67.0:0.5 (v/v) ammonia/methanol/THF with a 2.1 x 150 mm Zorbax Extend C₁₈ column using a) LC-MSD and b) HPLC-DAD to analyze a neat standard (30 μL injected).
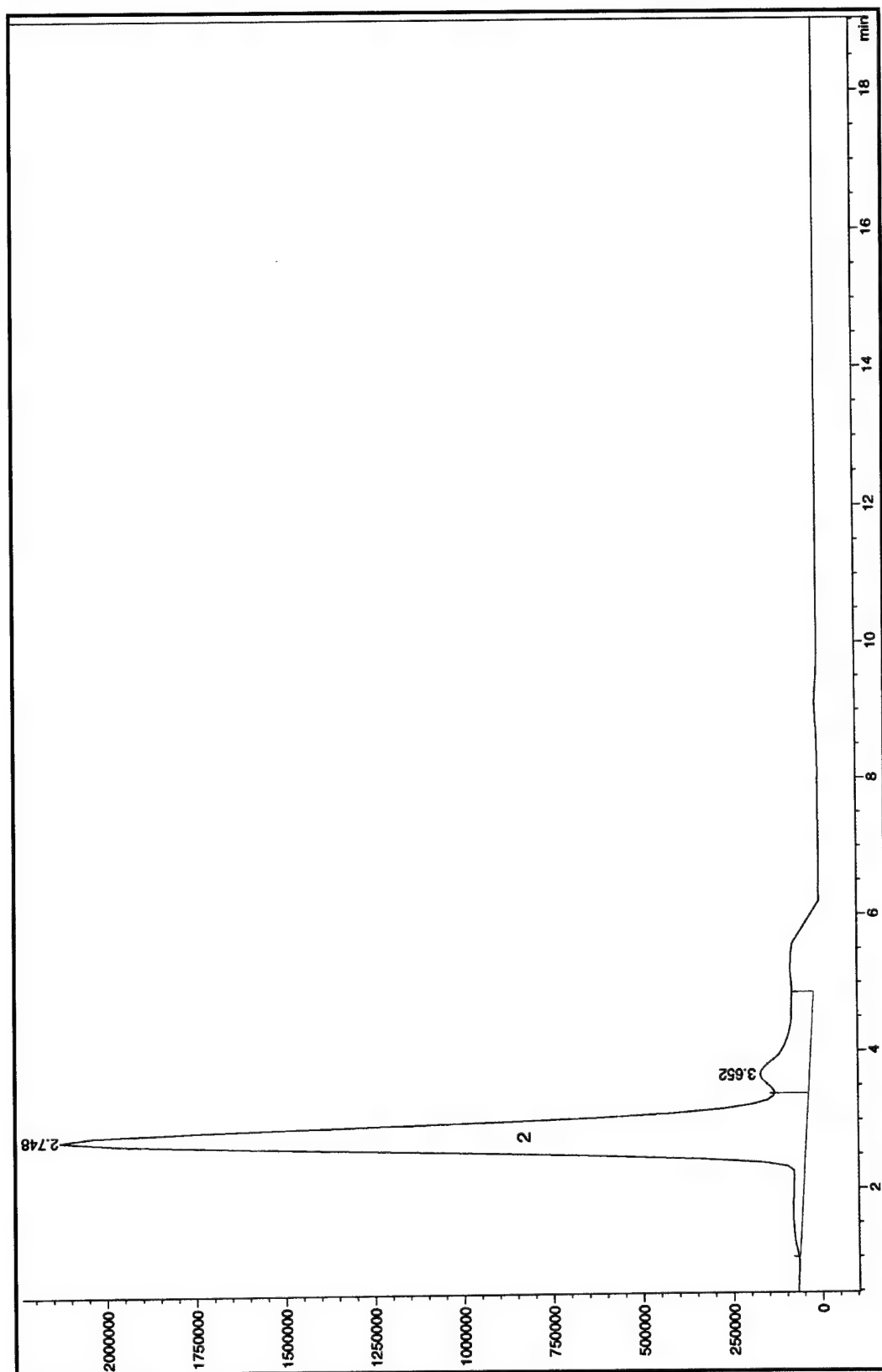
Figure 3.6 a). Total ion chromatogram obtained with a 2.1 x 50 mm Zorbax Extend-C_{18} column from a blood extract of blank blood containing 1 mg/L internal standard (trazodone, 2, 30 μL injected).

Table 3.9. Relative standard deviations (RSDs) of retention times for target drugs.

Compound	t_R	RRT[1]	RSD in t_R[1]
O-desmethylvenlafaxine	3.54	2.23	1.9
9-OH-risperidone	3.85	2.54	2.6
Risperidone	5.06	3.75	2.4
Citalopram	6.22	4.91	2.5
Fluvoxamine	8.40	7.09	6.0
Norfluoxetine	9.08	7.77	1.5
Venlafaxine	9.61	8.30	2.0
Paroxetine	11.07	9.77	0.6
Fluoxetine	14.69	13.38	0.34
Nefazodone	21.87	20.56	3.2
N-desmethylsertraline	22.68	21.37	4.2
Sertraline	35.49	34.18	2.5
Mean ± SD			2.5

[1] RRT = relative retention time; RSDs of retention times expressed in percent.

3.4. Discussion

As evidenced by these results, slight changes in mobile phase conditions can substantially alter retention and resolution. In chromatographic method development, the challenge was to optimise resolution while minimizing runtime. Compound elution from the chromatographic system can

be accelerated by selecting a mobile phase with physical and chemical properties more similar to the analytes than those of the stationary phase. Reasons for the effects of changing mobile phase parameters on compound resolution observed in this chapter are explored below.

3.4.1. *Effect of buffer choice on resolution*

Buffer choice can profoundly affect the degree of chromatographic separation. According to Kirkland, the most efficient and reproducible results from HPLC analyses of basic compounds can be obtained when using a pH equal to the buffer's $pK_a \pm 1$ (Kirkland, 1997). This suggests that ammonia and glycine should be most efficient as buffers at pH 8.2-10.2 and 8.7-10.7, respectively. Similarly, the best pH range for triethylamine should be 9.7-11.7. In the analyses carried out in this chapter, peak tailing of the selected psychiatric drugs was minimized and separation maximized when using $pH \geq pK_a+1$. This explains in large part why using the triethylamine-buffered mobile phases resulted in much lower compound resolution than mobile phases buffered with either ammonia or glycine. Using triethylamine as the buffer, it would be expected that the best separation would be achieved at $pH \geq 11.7$. At a pH of 10.0, less than optimal separation was realised.

Regardless of mobile phase composition, however, the chromatographic peaks for paroxetine and fluoxetine showed poorer peak symmetry than those for any other tested drugs (Figure 3.5). The reason for this may be that their molecular structures (see Table 1.3) result in reduced steric hindrance around the nitrogen atom in both drugs, thereby increasing the extent of interaction between compound and stationary phase. McCalley's studies provide direct experimental

evidence of these factors (McCalley, 1994, 1995). They have suggested that stereochemical effects may also influence peak shape, possibly by affecting penetration of the analyte to the column surface. That is, peak shape for some molecules with lower pK_a's may still be poor if overall molecular size is small and/or there is little steric hindrance around the nitrogen atom.

3.4.2. Modifying agent effect on resolution

One of the simplest ways of changing degree of analyte interaction with the mobile phase is to add a modifying agent. If only a binary mobile phase is used (i.e. ammonia/methanol), less polar compounds in the mixture become more difficult to elute, as both these solvents are polar. Table 3.4 lists the eluotropic strengths and polarity indices for the solvents tested in this chapter (Miller, 1987). In general, THF tended to decrease runtime, whereas AcN lengthened it. The reason for this is due to THF being less polar than methanol, whereas AcN is slightly more polar. Depending on the polarity of the analytes of interest, addition of either modifying agent might be advisable in other analyses.

Table 3.4. Eluotropic strengths and polarity indices of selected solvents[1].

Solvent	Eluotropic Strength	Polarity Index
Tetrahydrofuran (THF)	0.35	4.0
Methanol	0.73	5.1
Acetonitrile (AcN)	0.50	5.8
Water	Large	10.2

[1] After Miller (Miller, 1987).

3.4.3.   Effect of pH on resolution and base peak abundance

It was preferable to carry out liquid chromatographic separation of basic compounds, such as the psychiatric drugs in this dissertation, at high pH. Under these conditions they were mostly present in their free base form, minimizing unwanted ion-exchange interactions between them and the unreacted, completely ionised silanol groups in traditional C_{18} columns. However, mobile phase pH's greater than 7.0 have previously seen limited use, since the solubility of the stationary phase in such columns increases with increasing pH. This increased solubility means that the improved peak shape and column efficiency realized by analysing at high pH is cancelled out by the gradual degradation of the column's stationary phase. However, the advent of novel stationary phases such as that used in the Zorbax Extend-C_{18} column greatly minimize this concern, thus expanding the range of pH's which can be used. In the analyses carried out in this chapter, resolution sometimes changed drastically when small changes in pH were made, as in peak pairs 2, 4, and 5. It is interesting to note that peak pair 5 (venlafaxine, $pK_a = 9.24$ and norfluoxetine, $pK_a = 9.37$) was completely resolved at both pH 8.5 and 10.5, but coeluted at pH 9.5. Because each compound has a different pK_a, the elution order of some compounds changed with varying pH. If pH>pK_a of a basic compound, it will be present as a free base meaning its retention times will be lengthened due to decreasing ionisation. Peak symmetry of such compounds should be improved at high pH.

Increases in base peak abundance stemming from increasing pH (such as those observed in Figure 3.4) have also been observed in the high pH analysis of angiotensins using an ammonia-buffered mobile phase and electrospray ionisation in positive mode (Boyes, 2000). The reason

for this, however, is unclear. For all psychiatric drugs examined in this dissertation, the base

peak represents the molecular ion plus the weight of one proton (M+H) (see Table 3.1). For this

reason, selecting the best mobile phase pH in LC-MS analysis means finding a compromise

between obtaining maximum mass spectral peak abundance and sufficiently resolving analytes of

interest.

3.4.4. Differences in detection selectivity between DAD and MS

Despite incomplete resolution of all drugs, the monitoring of selected ions in an ion

chromatogram allows all drugs to be quantified. Mass spectrometry, being a more sensitive

detection method, imparts a larger signal than diode-array detection for some compounds. The

increased signal due to this enhanced sensitivity may, in some cases, hide the peak corresponding

to another closely eluting compound for which the ionisation conditions used are not as sensitive.

This results in apparent coelution. Such was the case with peaks 6 and 7 (venlafaxine and

norfluoxetine) as well as peaks 10 and 11 (N-desmethylsertraline and nefazodone). Since each

drug each has different molecular ions, they can be resolved using extracted ion chromatograms.

In summary, results from this work suggest HPLC-DAD or APESI-LC-MS analyses of

psychiatric drugs conducted using a methanolic mobile phase buffered with 0.01-0.05 M

ammonia to pH 10.0 will provide better retention and improved symmetry of peaks than

traditional mobile phases which employ acidic conditions. However, this requires the use of a

column such as the Zorbax Extend-C_{18}, which has a stationary phase that can withstand operating

at high pH. As of the writing of this dissertation, the method discussed in this chapter is the only

chromatographic method that detects all of the target psychiatric drugs (see section 1.8 for a review).

The use of isocratic elution is one major difference between the discussed method and most previously published LC-MS methods (Decaestecker et al, 2000; Muller et al, 2000b; Rittner et al, 2001; Tatsuno et al, 1996; Weinmann et al, 2000a). Precision, accuracy, and recovery for this method are comparable to those of earlier methods. The higher limit of quantitation (LOQ) for most drugs in this method compared to others is mainly due to two reasons. Firstly, most of the earlier-published methods used tandem mass spectrometry (MS/MS) (Cailleux et al, 1999; Decaestecker et al, 2000; Muller et al, 2000b; Weinmann et al, 2000a), which imparts much better detection sensitivity to a method than the use of conventional quandrupole MS (as was used in the discussed method). The LOQ's in methods that used quadrupole instruments were comparable to those in this method. Secondly, when using SIM mode, the greater the number of drugs included, the less sensitive the method becomes due to decreased dwell time for each selected ion. This method was used successfully to analyse all specimens collected at autopsy (blood, bile, brain, liver, urine, and vitreous humour) for psychiatric drug determination in other experiments conducted for this dissertation (see Chapters 5-7).

CHAPTER 4 : DRUG OF ABUSE ASSAY DEVELOPMENT

4.1. Introduction

The prevalence of substance abuse in people diagnosed with psychosis and/or depression has repeatedly been illustrated (Crum et al, 2001; Dhossche et al, 2000; Parker and Roy, 2001; Spak et al, 2000). This is cause for great concern as all of the commonly abused drugs affect one or more of the monoamine systems (see Section 1.3.4). It is therefore desirable to develop analytical methods capable of screening for a large range of drugs of abuse as well as the psychiatric drugs.

Each major class of the commonly abused drugs (amphetamine-like compounds, benzodiazepines, cannabis, cocaine, and opioids) is physicochemically diverse (see Tables 1.5 and 1.6). Because of these differences, most screening methods for drugs of abuse focus on a single category of compounds, often using class-specific immunoassay screens. Not only is this inefficient from the standpoint of requiring multiple screens for a single specimen, but additional analysis time and resources are required for separate screening methods for psychiatric drugs as well as any confirmations required for detected drugs. These confirmation techniques usually involve the use of either GC/MS or conventional HPLC (see section 1.8.2 for review). Developing a method that allows for screening and confirmation in one analysis would result in potential savings of both time and money.

This chapter describes development of a combined screening and confirmation method for drugs of abuse using similar LC-MS conditions to those for psychiatric drugs discussed in Chapter 3.

For optimum separation of the targeted drugs, slight modifications to this method were required. Three specimen preparation methods were also compared for use in combination with the optimised instrumental conditions. Instrumental parameters and mobile phase composition chosen for the determination of selected drugs of abuse in blood and urine are detailed, and the effect of changing different parameters on detectability is discussed.

4.2. Materials and methods

4.2.1. Materials and standards

Diazepam and oxazepam were obtained from the Division of Analytical Laboratories of the New South Wales Health Department (Lidcombe, Australia). All remaining drug standards were obtained from the Australian Government Analytical Laboratory (Southbank, Australia). Solvents were HPLC grade and were obtained from Sigma Aldrich Pty Ltd. Other chemicals were analytical reagent grade or better and were also obtained from Sigma Aldrich.

For the sake of simplicity, one drug from each class was used for initial method development: amphetamine, benzoylecgonine (BZE), flunitrazepam, and morphine. 5-(4-methylphenyl)-5-phenyl-hydantoin (MPPH) was used as the internal standard (IS). However, all drugs of abuse listed in Table 4.1 were used in method validation experiments. For methods development, 1

mg/mL stock solutions of standards were prepared monthly using methanol and stored at -20 °C until use. To enable a comparison of methods, 1 mg/L working standards were prepared fresh daily with deionised water.

Table 4.1. Drugs of abuse selected for method development and corresponding ions monitored in mass spectrometric detection[1].

Compound	Ions Monitored[2]		
	Quant ion	Qualifier 1	Qualifier 2
Amphetamine	**136** (100)	119 (40)	120 (0.48)
BZE	**290** (100)	291 (18)	292 (0.40)
Codeine	**300** (100)	301 (21)	302 (0.44)
Diazepam	**285** (100)	286 (21)	287 (36)
Flunitrazepam	**314** (100)	315 (20)	316 (0.45)
MDMA	**194** (100)	195 (15)	196 (0.39)
Methamphetamine	**150** (100)	119 (16)	120 (0.17)
Morphine	**286** (100)	287 (21)	289 (0.41)
Oxazepam	**287** (100)	289 (37)	290 (11)
Pseudoephedrine	**166** (100)	167 (13)	168 (0.14)
Temazepam	**301** (100)	302 (21)	303 (36)

[1] The m/z of the base peak for each drug is shown in bold.

[2] Relative ion abundances (expressed in %) shown in parentheses.

4.2.2. Details of extraction methods tested

Spiked blood specimens were prepared according to the experimental design shown in Table 4.2. The protein precipitation method currently used at the VIFM for HPLC analysis (Method A) was tested for suitability with LC-MS, and the results compared to those obtained using two different

published solid-phase extraction methods (Methods B and C). The methodology used in each technique is outlined in the following sections.

Table 4.2. Experimental design used for drug of abuse method optimisation.

Trial Method	Conditions
Protein Precipitation (Method A) (Drummer et al, 1993a)	250 µL blood specimen treated with 500 µL acetonitrile
Opioid SPE (Method B) (Gerastomoulos and Drummer, 1995)	Elution solvent: 30% 0.05 M NH$_4$OH (pH 9.0) in methanol; IS = MPPH[1]; other parameters the same
Amphetamine SPE (Method C) (Bogusz et al, 2000)	Specimens reconstituted in 55:44.5:0.5 ammonia/methanol/THF, pH 9.0; IS = MPPH[1]; other parameters the same

[1] MPPH = 5-(4-methylphenyl)-5-phenyl-hydantoin.

4.2.2.1. Method A

The specimen preparation method routinely used at the VIFM for drug screening using HPLC is a simple protein precipitation (Drummer et al, 1993a). Prior to specimen preparation, a 1 mg/mL solution of IS, MPPH, was diluted in 30 mL acetonitrile. For the protein precipitation, 250 or 500 µL of this mixture were added to a 2 mL plastic polypropylene tube (Sarstedt Group, Melbourne, Australia) containing a 250 µL aliquot of blood. This mixture was vortexed for 1 min, then centrifuged at 15,000 rpm in a Sorvall Microfuge (DuPont, Melbourne, Australia) for 10 min. The supernatant (30 µL) was then injected directly into the LC-MS.

4.2.2.2. Method B

The first solid-phase extraction method tested was based on one used at the VIFM for analysis of

opioids in blood (Gerastomoulos and Drummer, 1995).

Specimens (500 µL) were placed in 10 mL polypropylene extraction tubes (Selby-Biolab,

Clayton, Australia). The IS, MPPH (10 ng), and 2 mL sodium hydrogen carbonate buffer, pH

9.5, was added to each tube. This mixture was then frozen for approximately 5 min in a Dynavac

freeze bath at -30 °C (Dynavac, Auckland, New Zealand), then immersed in hot water to thaw

quickly. This procedure was repeated twice to disrupt cells, which can block solid-phase

cartridges. The specimens were centrifuged at 3500 rpm for 10 min and the supernatant

transferred to a clean polypropylene tube.

1-mL C_{18} Sep-Pak Vac cartridges (Millipore, North Ryde, Australia) were attached to a Varian

Vac Elut vacuum manifold (Varian Corp, Harbor City, California) capable of allowing 24

specimens to be prepared at the same time. Cartridges were conditioned with 2 mL methanol

followed by 2 mL deionised water and 1 mL 0.5 M sodium hydrogen carbonate buffer, pH 9.5.

Specimens were then applied to the cartridges, and the cartridges washed using 10 mL of 5 mM

ammonium sulphate buffer (pH 9.5). The cartridges were briefly dried under vacuum and the

compounds of interest eluted with 300 µL mobile phase. The eluate (30 µL) was then injected

into the LC-MS.

4.2.2.3. Method C

The second solid-phase extraction method tested was based on one published by Bogusz et al for the analysis of amphetamines without derivatisation (Bogusz et al, 2000). The IS, MPPH (10 ng), was added to a polypropylene extraction tube containing 500 µL of blood. The resulting mixture was vortexed briefly, then centrifuged for 5 min at 14,000 rpm. A 500 µL aliquot of the supernatant was transferred to another polypropylene tube to which 2 mL ammonium carbonate buffer, pH 9.3, was added. This mixture was then vortexed and centrifuged again for 10 min at 5000 rpm.

Bond Elute C_{18} cartridges (200-mg, Waters Australia Pty Ltd, Rydalmere, Australia), were attached to the vacuum manifold and conditioned with methanol (1 mL), deionised water (1 mL), and ammonium carbonate buffer, pH 9.3 (2 mL). The supernatant from the second centrifugation step was applied to the SPE cartridges, which were then rinsed with 2 mL of pH 9.3 ammonium carbonate buffer. The cartridges were briefly dried under vacuum for 5 min, and compounds of interest eluted using 500 µL of 9:1 methanol/0.5M acetic acid. 10 µL of 1.0 mmol HCl were added to the eluate, and this mixture was evaporated to dryness under nitrogen and reconstituted in mobile phase, 30 µL of which was injected into the LC-MS for analysis.

4.2.3. Instrumental conditions

LC-MS analysis using atmospheric pressure electrospray ionisation (APESI) was used for drug of abuse analyses. The psychiatric drug method outlined in Chapter 3 was used as the basis from

which to refine LC-MS parameters for the analysis of drugs of abuse in blood and urine.

Analysis was performed on a 1100 Series HPLC (Agilent Technologies, Forest Hill, Australia)

configured with a G1946A mass selective detector (MSD) operating in Selected Ion Monitoring

(SIM) mode.   The ions shown in Table 4.1 were monitored for compound detection. Generally,

monitored ions corresponded to the masses of protonated molecular and isotope ions. As

discussed in section 3.2.2.2, protonated isotope ions appear at reproducible ratios and can

therefore be used for drug quantitation.

The analytical system was linked to an IBM-compatible PC with ChemStation 4900 Series

software. Chromatographic separation was achieved using a Zorbax Extend-C_{18} column from

Agilent (2.1 x 150 mm, 5 μm particle size). The column was operated at 21 °C pumping at 0.25

mL/min.

The mobile phase used for psychiatric drug analyses did not provide sufficient separation of all

drugs of abuse. Therefore, to arrive at an improved separation, mobile phases comprising of

varying amounts of ammonia and methanol (keeping 0.5 % THF as a modifying agent) and

titrated to pH's ranging from 8.5 to 10.0 were tested on standards containing amphetamine, BZE,

flunitrazepam, and morphine.

To compare methods, resolution of each peak was calculated. Once a mobile phase had been

selected which adequately resolved these four compounds, the method was further tested on the

full set of test compounds listed in Table 4.1. An additional requirement that all compounds elute

in less than 15 min was imposed. The mobile phase that most efficiently separated the selected drugs comprised of 0.05 M ammonia/ methanol/THF (55:44.5:0.5), at pH 9.0.

4.2.4. Comparison of results

Analytical results were compared on the basis of drug recovery, intra- and inter-assay precision (measured as CV's), accuracy, and cleanliness of extracts. Blood specimens spiked with 0.75 mg/L standards of amphetamine, BZE, flunitrazepam, and morphine were extracted with each preparation method. Recovery was calculated by comparing the peak area of an extracted specimen to that of unextracted standards of the same concentration and expressed as a percentage. CV's were calculated using the standard deviation (σ) and mean (x) of 5 replicate analyses of specimens spiked with known concentrations using the formula CV = (σ/x)·100. Accuracy was calculated by dividing the measured concentration by the calculated concentration and multiplying the result by 100. Finally, extract cleanliness was evaluated by inspecting extracted ion chromatograms (EICs) from blank and spiked specimens.

This method was further tested for interfering peaks in actual cases. Blood and urine specimens from 11 coronial cases in which conventional HPLC and GC/MS had earlier detected compounds belonging to each class of drug of abuse were chosen for analysis using LC-MS. Cases in which several targeted compounds were present were preferentially chosen to test the applicability of the method to polydrug cases.

4.2.5. Statistical analyses

Statistical evaluation of this data was performed using SPSS V9.0.1 software on an IBM personal computer. Paired sample T tests at the 95 % confidence interval were used to determine if differences in drug recoveries observed in this chapter compared to those observed by other authors were statistically significant.

4.3. Results

As was observed in the psychiatric drug analyses discussed in chapter 3, changing pH and relative percent buffer concentration in the mobile phase had a profound effect on the chromatographic resolution of the selected drugs of abuse. Figure 4.1 shows the chromatographic separation of a number of drugs of abuse in an unextracted standard using the chromatographic parameters used to separate psychiatric drugs (see Section 3.3 to review these parameters). Slight modifications of these conditions were required for effective separation of drugs of abuse in postmortem specimens due to the presence of endogenous compounds. At pH 10, all test compounds had baseline resolution using a 0.05 M ammonia/methanol/THF (60:39.5:0.5) mobile phase. When standard solutions of the remaining test compounds (see Table 4.1) were analysed with this method, however, diazepam had a retention time of about 26 min.
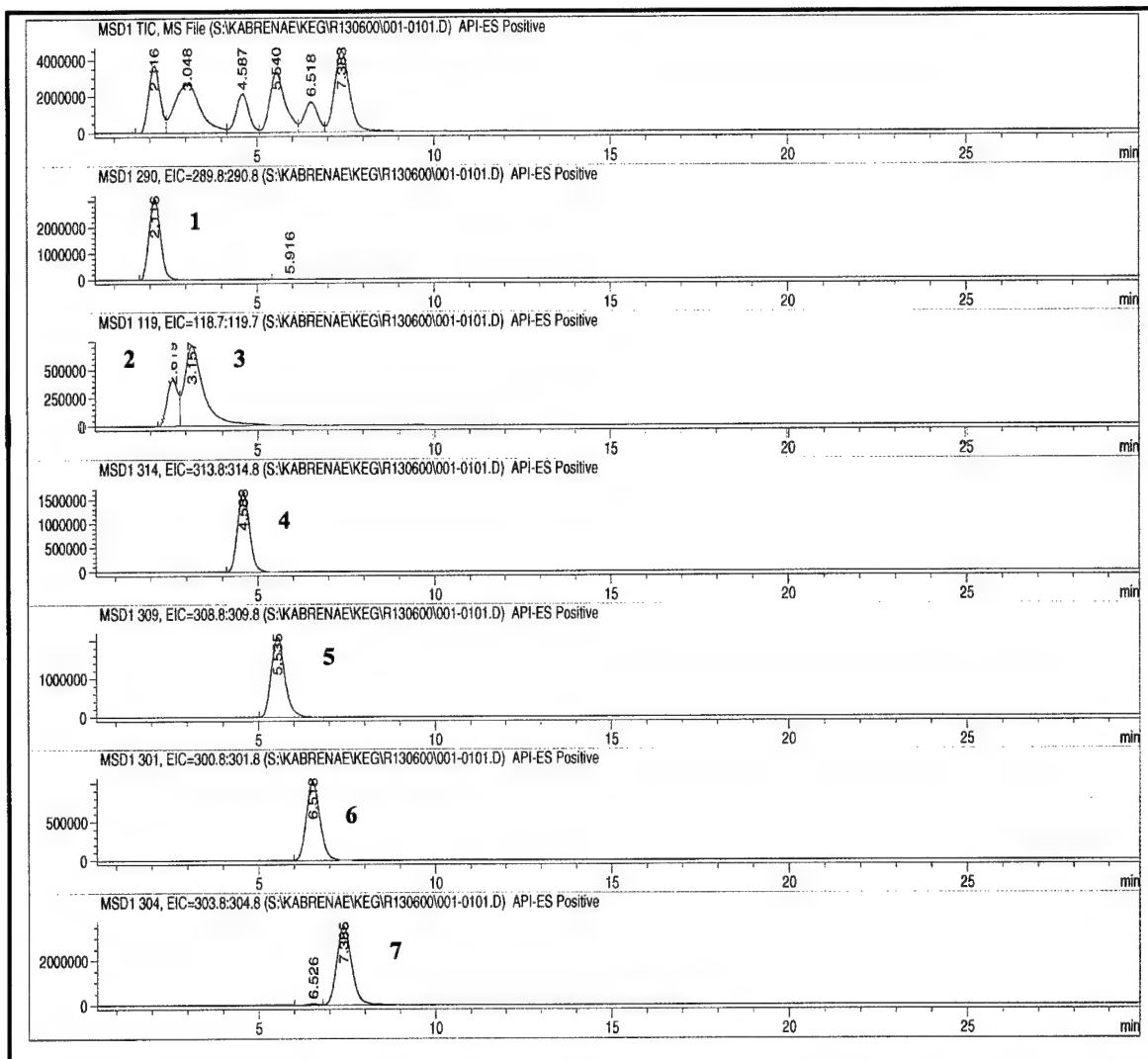
Figure 4.1. Chromatographic separation of an unextracted standard containing selected drugs of abuse separated using mass spectral parameters outlined in section 4.2.3., a Zorbax Extend-C$_{18}$ column (2.1 x 50 mm), and the mobile phase outlined in section 3.3 for separation of psychiatric drugs: 0.05 M ammonia/methanol/THF (67.0:32.5:0.5, pH 10.0). Drugs are identified as 1) BZE, 2) amphetamine, 3) methamphetamine, 4) flunitrazepam, 5) alprazolam, 6) temazepam, 7) cocaine (30 μL injected).

At pH 8.5, all test compounds eluted within 5 min but were not resolved, regardless of the relative ammonia/methanol composition in the mobile phase. This was not desirable, as competitive ionisation can occur when multiple compounds are present in the electrospray ionisation chamber, resulting in decreased detector sensitivity. Compound resolution is still important in LC-MS, even with the capability of using EICs for quantitation. An ammonia/ methanol combination at pH 9.0 was chosen as the optimum mobile phase. This was then used to assess the three specimen preparation methods.

Accuracy, precision, and drug recoveries are shown in Table 4.3. Method A had the highest recoveries but the lowest precision (CV's>10% for all test drugs). The lowest recoveries were observed with method B, although it had the best precision. Method C, which had the second highest recoveries out of the three methods overall, provided accuracy within the required range and the required reproducibility (CV ≤ 20%) for all test compounds.

Table 4.3. Mean recoveries, accuracy, and precision data (expressed as percent) for trial specimen extraction procedures[1-3].

Compound	Rec	Acc	Inter-assay CV	Intra-assay CV	Rec	Acc	Inter-assay CV	Intra-assay CV	r^2	Rec	Acc	Inter-assay CV	Intra-assay CV
Amphetamine	110	120	13	18	58	65	8	7	0.996	47	120	18	10
BZE	51	110	84	120	22	110	9	14	0.997	120	120	14	19
Codeine[1]									0.998	60	94	13	6
Diazepam[1]									0.992	78	90	11	9
Flunitrazepam	67	84	35	17	6	110	5	9	0.999	92	120	3	19
MDMA[1]									0.997	59	95	16	7
Methamphetamine[1]									0.992	40	92	10	3
Morphine	85	120	11	11	55	100	19	13	0.994	31	120	7	14
Oxazepam[1]									0.999	67	110	10	18
Pseudoephedrine[1]									0.993	58	91	12	7
Temazepam[1]									0.998	60	93	16	9

[1] Codeine, diazepam, MDMA, methamphetamine, oxazepam, pseudoephedrine, and temazepam were used to validate the final extraction and analysis method in death investigation cases, but were not included in the original specimen preparation method comparison. [2] n=5 for each extraction method tested. [3] Blank blood specimens spiked with 0.075 mg/L standards of target drugs of abuse for method validation.

Figure 4.2 shows chromatograms of blank extracts using each extraction method. Method A (Drummer et al, 1993a) did not produce sufficiently clean extracts. Interfering peaks were observed in the EIC for MPPH, morphine, and amphetamine. For LC-MS analysis, it was found that increasing the volume of acetonitrile/MPPH solution to 500 μL provided a cleaner specimen, but interfering peaks were still present. For this reason, this method was not pursued further. Method B (Gerastomoulos and Drummer, 1995) produced extracts free from interfering peaks, although low drug recoveries reduced the detectability of some compounds present in specimens at trace concentrations (Table 4.3). Of the three preparation methods, method C (Bogusz et al, 2000) performed the best on all measures (accuracy within ±20%, CV's ≤ 20%, and the least interfering compounds in extracted ion chromatograms). It was therefore chosen as the preferred specimen preparation method for further validation work.

Although both Δ^9-THC and Δ^9-THC-COOH were initially included in this research, neither could be chromatographed using any of the chosen mobile phases. Therefore, both compounds were excluded from further method development. A representative set of chromatograms resulting from LC-MS analysis of selected drugs of abuse extracted using method C is shown in Figure 4.3. For quantitation purposes, extracted ion chromatograms (EICs) were selected after analysis. The limit of quantitation for mass spectral detection, defined in the same manner as for psychiatric drug analyses, was 0.05 mg/L for all drugs. Calibration curves passed through the origin and were linear to at least 5.0 mg/L with r^2 values of 0.99 or better (see Table 4.3).
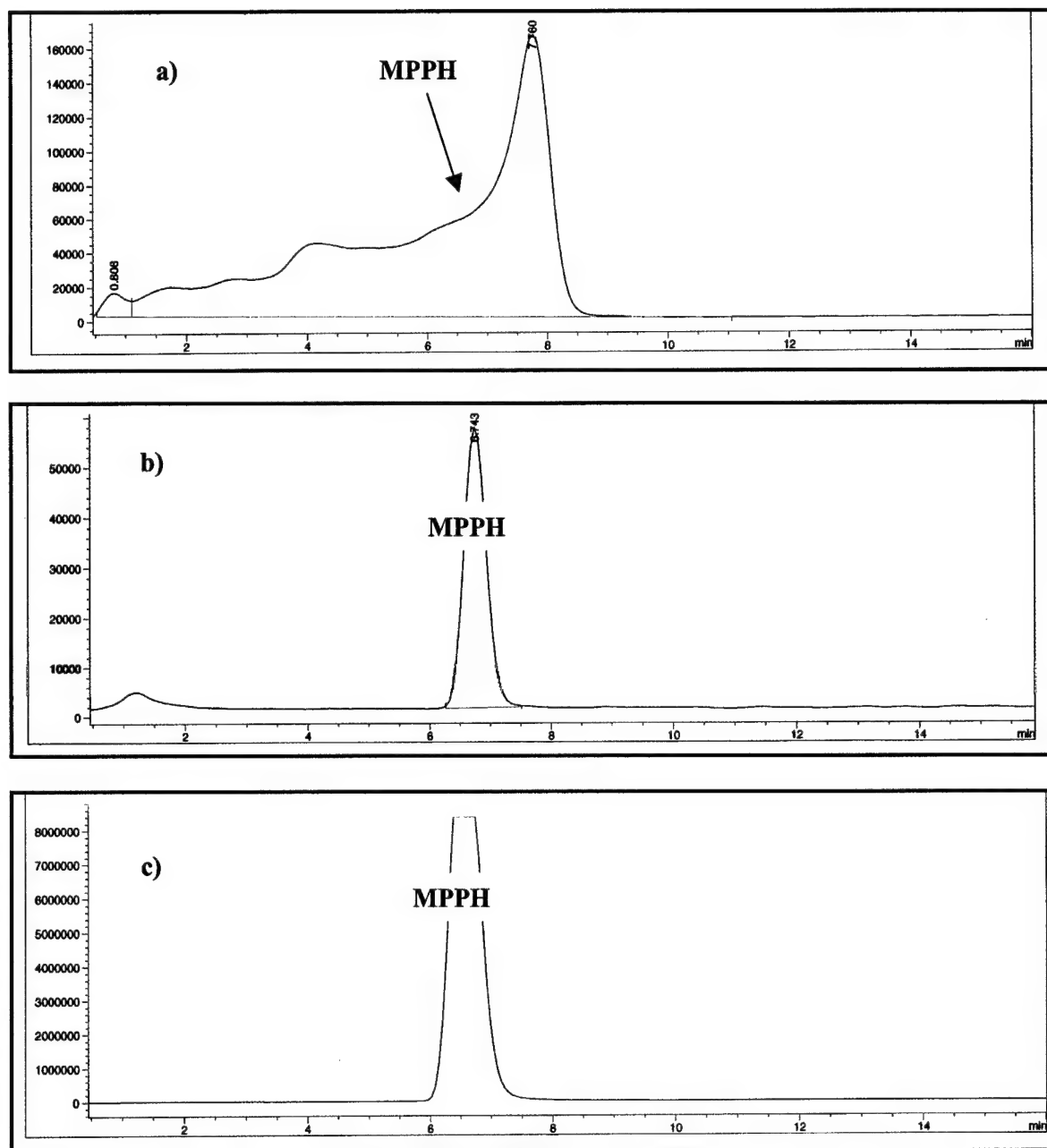
Figure 4.2. Extracted ion chromatograms (m/z 267) of blank blood specimens spiked with MPPH (IS) using mass spectral parameters outlined in section 3.2.2.2, a Zorbax Extend-C$_{18}$ column (2.1 x 50 mm), mobile phase consisting of 0.05 M ammonia/methanol/THF (44:54.5:0.5, pH 9.0), and extracted using a) Method A, b) Method B, and c) Method C (30 µL injected).
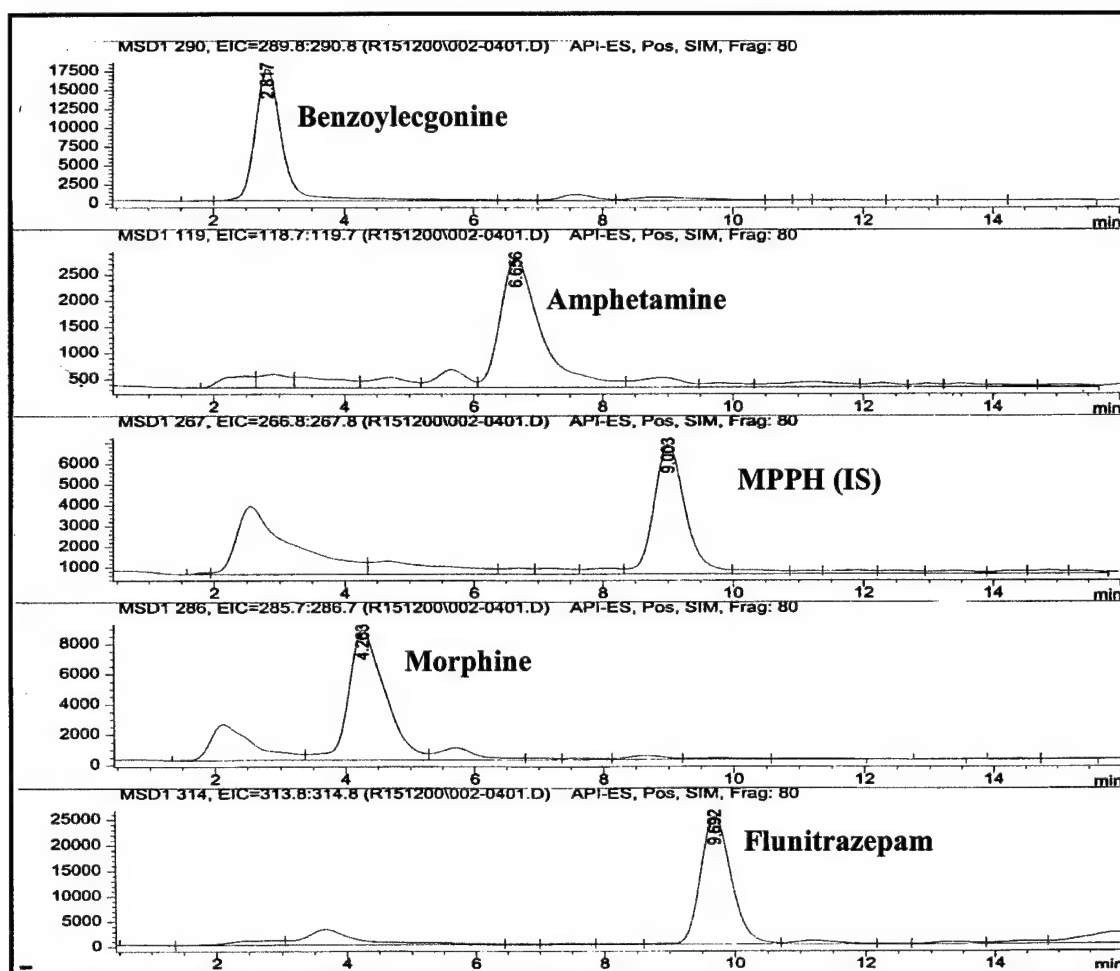
Figure 4.3. LC-MS separation of selected drugs of abuse in blood extracted using method C, mass spectral parameters outlined in section 4.2.3 and injecting 30 μL, Zorbax Extend-C$_{18}$ column (2.1 x 150 mm), and a mobile phase consisting of 0.05 M ammonia/methanol/THF (44:54.5:0.5, pH 9.0).

The use of this method has been extended to the detection of over 40 basic drugs using the developed LC-MS conditions (Table 4.4.). Although the retention times of some of the late-eluting compounds were quite long using this method, they could be shortened by using a gradient method or a different mobile phase which focused on these late eluting drugs.

Table 4.4. Drugs detected in standards using the LC-MS conditions described in this chapter[1].

Compound	RT	Compound	RT	Compound	RT
Cotinine	0.53	Oxycodone	7.99	Nortriptyline	31.10
7-NH$_2$-nitrazepam	1.47	MPPH (IS)	8.81	Chlorpheniramine	41.41
7-NH$_2$-clonazepam	1.51	Moclobemide	8.84	Quinine Sulphate	47.57
7-NH$_2$-flunitrazepam	2.27	Pholcodine	8.92	Citalopram	48.47
BZE	2.62	Strychnine	9.54	Sertraline	50.97
Phentermine	3.20	Flunitrazepam	9.69	Desipramine	58.01
MDMA	3.38	Prochlorperazine	12.29	Olanzapine	63.02
Morphine	3.61	Nitrazepam	14.56	Paroxetine	63.11
Pseudoephedrine	4.11	Chlorpromazine	16.83	Benztropine	80.62
Nordiazepam	4.39	Fenfluramine	20.19	Ephedrine	81.71
Diazepam	5.67	Triazolam	22.28	Bupivacaine	142.62
Trifluoperazine	5.94	Alprazolam	22.97	Haloperidol	148.40
Amphetamine	6.66	Temazepam	27.61	Doxepin	197.27
Codeine	6.72	Propranolol	28.43	Promethazine	258.98
Methamphetamine	7.56	Venlafaxine	29.04	Mianserin	288.87
Hydrocodone	7.89	Cocaine	30.96		

[1] Retention times expressed in min. Compounds eluted using a Zorbax Extend-C$_{18}$ column (2.1 x 150 mm) and mobile phase consisting of 0.05 M ammonia/methanol/THF (44:54.5:0.5, pH 9.0).

Blood and urine specimens from 11 coronial cases positive for different drugs of abuse are detailed in Table 4.5. Those obtained using conventional techniques adopted by the VIFM toxicology laboratory are shown in parentheses. Analytical results obtained using LC-MS generally compared favourably to the original results. The comparatively higher sensitivity of LC-MS compared to the conventional techniques used in the laboratory enabled detection of drugs present even at trace concentrations.

Table 4.5. Toxicological results from blood and urine of 11 coronial cases[1-3].

Compound	1	2	3(U)	3	4	5	6	7	8	9	10	11(U)	11
Amphetamine	–	0.15 (0.12)	120 (18)	0.60 (0.08)	<0.05 (ND)	–	–	–	–	–	–	–	–
BZE	–	–	13 (33)	0.05 (<0.05)	–	–	–	–	–	–	–	150 (114)	0.99 (0.4)
Codeine	–	–	7.0 (5.5)	–	–	–	–	1.0 (1.2)	–	–	–	–	–
Diazepam	–	–	–	–	–	–	0.49 (0.6)	0.53 (0.3)	–	–	–	–	–
Methamphetamine	–	–	–	0.40 (0.1)	0.25 (0.2)	–	–	–	–	–	–	0.05 (Det[1])	<0.05 (ND[1])
Morphine	–	–	–	–	–	–	–	0.08 (0.05)	0.15 (0.10)	0.05 (0.05)	0.18 (0.17)	–	–
Oxazepam	–	–	–	–	–	0.66 (0.49)	0.19 (0.09)	1.9 (1.19)	–	–	–	–	–
Pseudoephedrine	0.70 (0.6)	–	–	–	–	–	–	–	–	–	–	–	–
Temazepam	–	–	–	–	–	0.94 (0.7)	0.21 (0.1)	0.89 (0.7)	–	–	–	–	–

[1] Results from urine specimens annotated by a "U". Concentrations expressed in mg/L. Det = detected but not quantitated, ND = not detected. [2] Concentrations determined in original analyses shown in parentheses. [3] Cases include 5 natural deaths (cases 1, 6, 8-10), 4 accidental deaths (cases 3-5 & 7), 1 suicide (case 2) and 1 homicide (case 11).

4.4. Discussion

It was anticipated that drug recoveries using extraction Method A would be high and that extracts would be dirty, as this method is essentially just a protein precipitation step. Similarly, the comparatively low drug recoveries obtained using Method B could also be expected because of the large volume of buffer used in the cartridge wash step. In addition to removing impurities, the extensive washing also may have caused certain compounds of interest to be washed out. Thus, while amphetamine and morphine, with pK_a's of 9.9 and 8.1, respectively, were retained on the SPE cartridge using pH 9.5 buffer washes, BZE and flunitrazepam (pK_a's = 3.8 and 1.8, respectively) were much less retained. The lack of interfering peaks in extracts prepared with Method B compared to Method A, however, helped increase assay precision.

Results from analyses of selected coronial cases (Table 4.5) show that each drug could be quantitated without observable interference using Method C. As with the psychiatric drug analyses (section 3.3), the use of extracted ion chromatograms enabled determination of the drugs present in the 11 cases even when peaks were incompletely resolved in the total ion chromatogram. In the case of amphetamine and methamphetamine, which differed in retention time by only 0.9 min, monitoring separate isotope ions (m/z 136 for amphetamine and 150 for methamphetamine) as qualifier ions made it possible to differentiate between the two compounds.

Although this method was not able to detect either Δ^9-THC or Δ^9-THC-COOH due to their much higher lipophilicity, it was useful for the determination of a number of other common drugs of

abuse. Additionally, the LC-MS parameters used in this chapter have been demonstrated in the detection of over 40 basic drugs in standard mixes. Based on the results shown in Figure 4.1, it appears that standard solutions containing many of the target drugs of abuse could have been adequately separated within 15 min using the same mobile phase as for the detection of psychiatric drugs (see Chapter 3). However, when used in conjunction with the tested extraction methods, endogenous compounds coeluted with MPPH, morphine, amphetamine, and methamphetamine, which interfered with the quantitation of these compounds. The use of an instrument with MS/MS capability would have provided the ability to discriminate between target compounds and endogenous peaks, thereby allowing the use of the same conditions for psychiatric drugs and drugs of abuse alike.

The ability of LC-MS to 1) scan for multiple drugs of abuse simultaneously, and 2) to perform compound confirmation in the same run are the major advantages its use affords compared to conventional immunoassay, which targets classes of drugs in urine (amphetamines, cocaine metabolites, benzodiazepines, cannabinoids, and opiates). The ability to perform screening and confirmation in one run results not only in reduced analysis time, but also decreased costs, and a reduction in the volume of specimen required.

Most previously published LC-MS methods that target multiple classes of drugs of abuse did not target amphetamine-like compounds (Bogusz et al, 1998b; Cailleux et al, 1999; Decaestecker et al, 2000). Benzodiazepines were not targeted in two methods (Cailleux et al, 1999; Tatsuno et al, 1996). Decaestecker et al presented a method which used the ability of a quadrupole time-of-flight (Q-TOF) detector to switch between single and tandem mass spectrometry for quantitative

profiling of selected benzodiazepines and opioids, cocaine and metabolites, MDMA, and a small number of other psychoactive drugs in a "general unknown" specimen (Decaestecker et al, 2000). Their method employed gradient elution, which resulted in relatively longer runtimes ($t_R = 24.43$ min for trazodone, the latest-eluting compound, with an additional 4 min required between runs for re-equilibration) in comparison to the method described in this chapter.

A robust method developed by Rittner et al enabled the combined screening and confirmation of 70 psychoactive drugs including amphetamine-like compounds, benzodiazepines, and some opioids in serum (Rittner et al, 2001). This method used collision-induced dissociation (CID) with electrospray ionisation in positive mode to enhance detection sensitivity. However, like that of Decaestecker et al's, this method employed gradient elution with a longer overall runtime than that described in this chapter.

The methods developed in the laboratories of both Cailleux et al and Bogusz et al still used a somewhat class-specific approach. Bogusz et al's method required five separate isocratic mobile phases for the determination of all target compounds, although each consisted of the same two components so switching between each was simplified (Bogusz et al, 1998b). Additionally, two different fragmentor voltage settings were required -- one for cocaine, BZE, LSD, ibogaine, and dihydrocodeine, and one for the remaining compounds. Similarly, Cailleux et al's method required two mobile phases and fragmentor voltage settings: one for opioids and one for cocaine and its metabolites (Cailleux et al, 1999). Although all compounds eluted in less than 9 minutes in both methods, additional time for column equilibration and replicate analyses again resulted in longer overall runtime needed for a given set of case samples.

Cailleux et al reported lower LOQs than those reported for the present method, most likely due to their use of tandem mass spectrometric detection. Tatsuno et al, who used thermospray ionisation in a single-quadrupole instrument, also reported lower LOQs for analysis of their target compounds in urine (Tatsuno et al, 1996). In their method, they used gradient elution, decreasing vaporiser temperature linearly in accordance with changing mobile phase composition to maximise sensitivity.

Bogusz and colleagues have applied their SPE method (Method C) to the determination of amphetamine and related compounds, opiate agonists, cocaine and metabolites, flunitrazepam and metabolites, and LSD in biological specimens, although they have not reported simultaneous analysis of the various classes of drugs in one run (Bogusz et al, 1997a; Bogusz et al, 1997b; Bogusz et al, 1998a; Bogusz et al, 1998b; Bogusz et al, 2000). In their analyses, they used atmospheric pressure chemical ionisation (APCI), which they cite as being more sensitive for most compounds in comparison to atmospheric pressure electrospray ionisation (APESI). The LOQs reported in this chapter using APESI, however, are well within the range of limits of detection reported for drugs of abuse in these methods (0.1-200 ng/mL). This method was able to detect a range of commonly abused drugs of abuse at concentrations typically observed in the postmortem or clinical setting. Detection sensitivity and specificity could, however, be enhanced through the use of an ion-trap mass spectrometer or triple quadrupole instrument, which would provide MS-MS capability (Marquet, 2002).

Table 4.6 shows drug recoveries obtained in drug of abuse analyses discussed in this chapter compared to those reported by Bogusz et al (Bogusz et al, 1997a; Bogusz et al, 1997b; Bogusz et

al, 1998a; Bogusz et al, 2000). Bogusz' team achieved drug recoveries for opiate agonists and amphetamines which were higher, on average, than those obtained for this drug class in my analyses. This was not the case, however, for other drug classes.

Table 4.6. Drug recoveries (%) obtained using method C with the LC-MS parameters outlined in this chapter and those obtained by Bogusz et al[1].

Drug	This Chapter	Bogusz et al[1]
Amphetamine	47	86
BZE	120	no results
Codeine	60	88
Diazepam	78	no results
Flunitrazepam	92	92
MDMA	59	90
Methamphetamine	40	82
Morphine	31	85
Oxazepam	67	no results
Pseudoephedrine	58	58
Temazepam	60	no results

[1] (Bogusz et al, 1997a; Bogusz et al, 1997b; Bogusz et al, 1998a; Bogusz et al, 2000).

These results indicate that, with slight modifications, an LC-MS method developed for the determination of psychiatric drugs in postmortem specimens can also be used to detect and quantitate commonly abused drugs in the same specimens. The capability for screening and confirming multiple drugs belonging to different classes greatly expands the utility of LC-MS.

CHAPTER 5 : TISSUE DISTRIBUTION OF

PSYCHIATRIC DRUGS

5.1. Introduction

Many drugs are found at higher concentrations in the solid tissues (such as heart, lung, and liver) than in blood. The manner in which a drug distributes in life is dependent on a number of factors, including its lipophilicity, pK_a, molecular weight, and its affinity for particular tissues. It is therefore important to understand the pattern of a drug's distribution in tissues to be able to determine any potential tissue-specific toxicity and to properly evaluate the likelihood of redistribution occurring postmortem.

Knowledge of a drug's distribution pattern is also useful in death investigations when comparing its concentrations in different tissues. Liver specimens, for example, are often obtained and analysed for drug content when blood or other tissues are unavailable due to putrefaction, extensive injury, or exsanguination (Coe, 1977; Drummer, 2001; Sturner et al, 2000). It is therefore important to determine if any correlation exists between drug concentrations in liver and blood. Vitreous humour specimens can also be valuable for toxicological testing as they are less likely to be subject to putrefactive changes (Bost, 1997; Coe, 1977).

Studies of the tissue distribution of psychiatric drugs were reviewed in section 1.5.1. These studies showed some common trends for different drugs, although there were some differences in

patterns of distribution. Since each of these studies looked only at a single psychiatric drug, these trends and differences are easily overlooked. Additionally, these studies did not use the same tissues for analysis, further complicating the comparison of those patterns reported in each study. Therefore, in order to better understand the distribution of psychiatric drugs as a class, a variety of tissue specimens collected at autopsy during the investigation of deaths occurring in the state of Victoria were analysed for the target psychiatric drugs and certain metabolites (Table 3.1).

5.2. Experimental

5.2.1. Specimen preparation

Cases were selected and ethics approval gained in accordance with protocols outlined in Chapter 2. Specimens were collected and prepared using the methods outlined in the same chapter. In total, specimens from 85 death investigation cases were collected. The metabolites of fluoxetine, risperidone, sertraline, and venlafaxine were measured as well as the parent drug.

5.2.2. Instrumental conditions

Instrumental analysis of prepared specimens was performed using the instrumental conditions outlined in Chapter 3. For quantitation purposes, mass spectral detection in SIM mode was conducted, monitoring the ions listed in Table 3.1.

5.2.3. Statistical analyses

Statistical evaluation of this data was performed using SPSS V9.0.1 for Windows software on an IBM personal computer. Parametric Pearson correlation tests at the 95% confidence interval were used to determine correlation of tissue to blood concentrations for each drug.

5.2.4. Comparison of tissue:blood ratios to published studies

Tissue:femoral blood concentration ratios in published studies of the tissue distribution of the psychiatric drugs under investigation were calculated. These ratios were compared to those in the cases included in this chapter.

5.3. Results

5.3.1. Sertraline

5.3.1.1. Tissue concentrations of sertraline -- summary

There were 21 sertraline-positive cases studied in this dissertation. Individual data plus mean, range, and median tissue concentrations of both sertraline and N-desmethylsertraline are displayed in Table 5.1. The highest mean concentration of sertraline was detected in liver, followed by bile, frontal cortex, and femoral blood. Sertraline concentrations in vitreous humour

were the lowest. The mean N-desmethylsertraline to sertraline ratio was 9.5:1, and ranged from 0.03-300:1. N-desmethylsertraline concentrations in blood were significantly correlated with those of sertraline ($r^2 = 0.514$, p<0.01).

5.3.1.2. Sertraline tissue:blood concentration ratios

The ratios of the mean concentrations of sertraline and N-desmethylsertraline in tissue to femoral blood are shown in Figure 5.1. The highest concentration ratios were observed with liver, while the vitreous humour concentration ratio was the lowest.
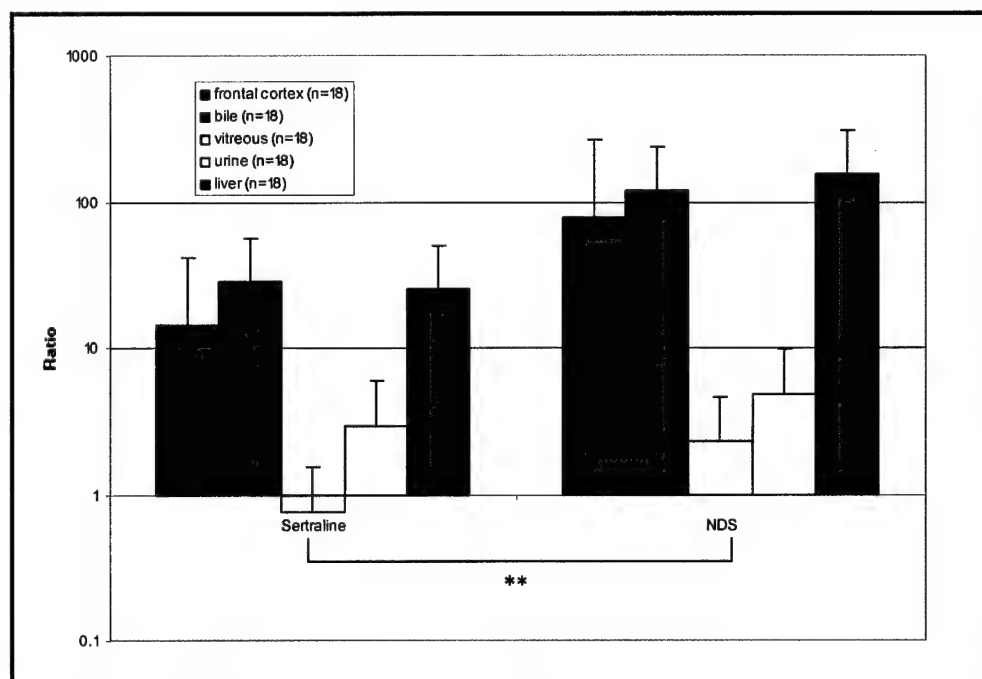
*Figure 5.1. Concentration ratios of sertraline and N-desmethysertraline (NDS) in postmortem tissues compared with femoral blood (means ± SD's). ** p<0.01.*

Table 5.1. Summary of sertraline and N-desmethylsertraline tissue concentrations[1,2].

Case No.	Blood (F) (n=21)	Frontal Cortex (n=16)	Bile (n=16)	Vitreous (n=18)	Urine (n=18)	Liver (n=18)
5	<0.05 (<0.05)	5.7 (38)	20 (60)	<0.05 (<0.05)	0.76 (3.1)	6.1 (27)
6	<0.05 (0.34)	1.8 (4.8)	3.3 (99)	0.13 (0.91)	1.4 (1.1)	3.3 (13)
7	<0.05 (<0.05)	0.73 (5.2)	<0.05 (<0.05)	0.11 (<0.05)		0.62 (4.7)
8	0.14 (0.22)	2.7 (100)	1.3 (40)	0.14 (1.1)	0.14 (0.74)	2.7 (1.1)
10	0.48 (0.23)				<0.05 (0.10)	
13	0.83 (<0.05)			0.18 (0.22)		51 (92)
17	1.0 (0.70)	17 (12)	1.7 (1.7)	0.20 (0.47)	3.2 (0.95)	18 (16)
21	0.99 (1.3)	1.0 (0.12)	9.3 (10)	0.15 (0.27)	0.30 (0.37)	2.5 (8.8)
25	0.05 (0.38)	<0.05 (3.9)		<0.05 (<0.05)		0.63 (13)
26	0.35 (2.0)	0.94 (2.6)	1.2 (83)	0.13 (0.14)	0.57 (4.0)	1.8 (9.5)
27	0.46 (0.56)	0.83 (21)	<0.05 (0.15)	<0.05 (0.54)	<0.05 (<0.05)	20 (61)
28	2.7 (5.6)	2.2 (11)	2.1 (56)	<0.05 (<0.05)	0.20 (0.54)	18 (26)
23	0.24 (0.42)					
24		2.7 (0.63)	<0.05 (<0.05)	<0.05 (<0.05)	0.24 (<0.05)	0.20 (0.57)
34	<0.05 (<0.05)	0.47 (0.21)	0.30 (5.5)	0.14 (1.1)	<0.05 (0.33)	0.11 (2.1)
35	0.31 (<0.05)		13 (6.3)		0.63 (0.27)	
36	0.32 (0.18)		3.1 (16)	0.15 (0.50)	0.69 (1.6)	18 (34)
37	1.3 (<0.05)	0.50 (5.7)	<0.05 (15)	0.17 (0.22)	<0.05 (<0.05)	0.14 (4.6)

Case No.	Blood (F) (n=21)	Frontal Cortex (n=16)	Bile (n=18)	Vitreous (n=18)	Urine (n=18)	Liver (n=18)
38	1.6 (1.5)	0.73 (36)			0.76 (0.42)	110 (74)
47	<0.05 (0.06)	2.2 (0.06)		0.06 (0.06)	0.08 (0.07)	<0.05 (<0.05)
48	0.79 (0.54)		7.1 (7.9)	<0.05 (<0.05)	0.71 (0.41)	
50	31 (2.1)	0.97 (0.52)	<0.05 (<0.05)	0.09 (0.10)	0.06 (<0.05)	0.22 (0.09)
Mean ± SD	0.54 ± 0.66 (0.75 ± 1.3)	2.5 ± 4.0 (14 ± 25)	3.7 ± 5.6 (24 ± 32)	0.11 ± 0.05 (0.31 ± 0.36)	0.54 ± 0.74 (0.75 ± 1.1)	13 ± 27 (20 ± 27)
Range	<0.05-31 (<0.05-5.6)	<0.05-5.7 (0.06-100)	<0.05-13 (<0.05-99)	<0.05-0.18 (<0.05-1.1)	<0.05-3.2 (<0.05-4.0)	<0.05-110 (<0.05-92)
Median	0.32 (0.34)	1.0 (4.8)	1.3 (7.9)	0.11 (0.14)	0.24 (0.37)	2.5 (9.5)

[1] F = femoral; H = heart. [2] All concentrations in mg/L or mg/kg; metabolite concentrations expressed in parentheses.

5.3.1.3. Correlation of blood sertraline and N-desmethylsertraline concentrations

to those in other tissues

Liver sertraline concentrations exhibited the highest and only statistically significant correlation with blood (r^2 = 0.231), although this was relatively low. N-desmethylsertraline concentrations in liver showed no significant correlation with blood (Table 5.2, see also Figure 5.2).

Table 5.2. Sertraline correlation data of postmortem tissues compared to femoral blood[1,2].

Tissue	Pearson Correlation (r^2)	95% confidence interval for m	p-value
Liver	**0.231**	**0.000-0.025**	**0.043**
	(0.002)	(-0.024-0.029)	(0.868)
Frontal Cortex	0.011	-0.088-0.127	0.701
	(0.011)	(-0.038-0.026)	(0.704)
Vitreous	0.009	-5.637-8.120	0.707
	(0.066)	(-2.865-0.952)	(0.304)
Urine	0.007	-0.415-0.571	0.743
	(0.006)	(-0.562-0.747)	(0.768)
Bile	0.004	-0.080-0.061	0.769
	(0.084)	(-0.011-0.037)	(0.277)

[1] Significant correlations in boldface. [2] Metabolite correlation data expressed in parentheses.
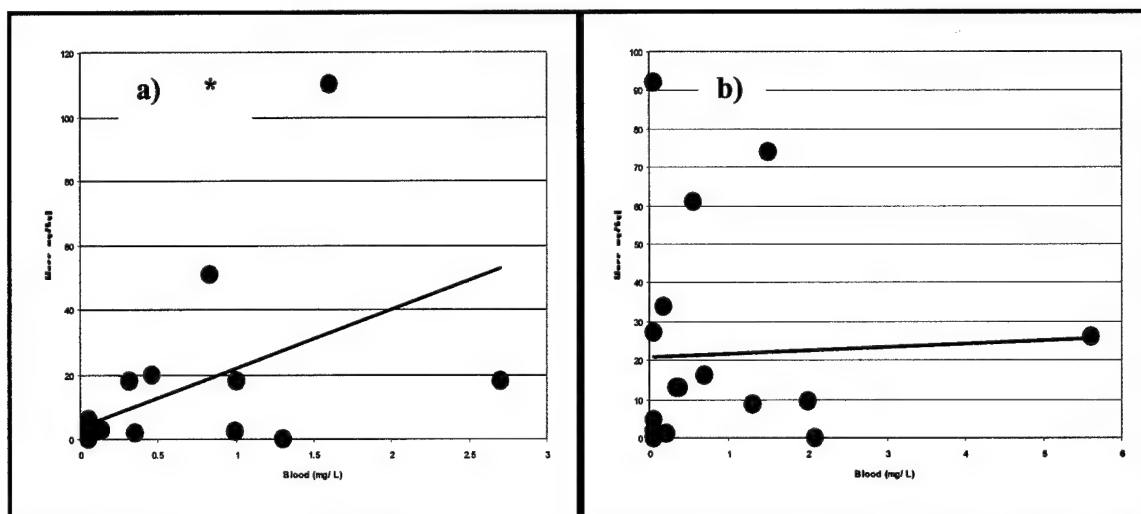
*Figure 5.2. Scattergrams showing a) slight positive correlation between liver and femoral blood sertraline concentrations and b) poor correlation between liver and femoral blood N-desmethylsertaline concentrations. * p<0.05.*

5.3.2. Venlafaxine

5.3.2.1. Tissue concentrations of venlafaxine -- summary

Table 5.3 shows postmortem tissue concentrations from 20 cases that tested positive for venlafaxine. The highest mean venlafaxine concentrations were found in urine, followed by frontal cortex, bile, and liver. The lowest mean concentrations were detected in vitreous humour. The ratio of O-desmethylvenlafaxine to venlafaxine was similar for each tissue (averaging 2.8:1), although it varied from 0.03-250:1. Like N-desmethylsertraline, blood concentrations of O-desmethylvenlafaxine showed a small but significant correlation with those of venlafaxine (r^2 = 0.330, p<0.01).

Table 5.3. Summary of venlafaxine and O-desmethylvenlafaxine tissue concentrations[1,2].

Case No.	Blood (F) (n=20)	Frontal Cortex (n=9)	Bile (n=17)	Vitreous (n=17)	Urine (n=16)	Liver (n=16)
14	0.20 (<0.05)		<0.05 (0.66)	0.19 (0.26)		<0.05 (0.64)
52	0.19 (0.08)	0.40 (<0.05)	19 (14)	0.47 (0.76)	2.4 (5.1)	2.9 (2.1)
53	1.1 (1.4)	0.31 (0.21)	2.2 (4.9)	1.2 (0.93)		0.85 (1.6)
55	0.07 (0.67)	1.3 (5.9)	1.4 (1.5)	0.34 (0.84)	0.19 (0.14)	1.4 (1.0)
57	8.5 (0.52)	4.6 (16)	4.2 (18)	1.8 (3.4)	37 (250)	1.8 (10)
59	0.12 (1.7)			0.63 (1.0)	30 (23)	0.31 (0.69)
60	0.24 (3.4)	0.90 (2.9)	8.7 (6.8)	0.55 (0.81)	16 (30)	2.8 (7.3)
61	6.6 (2.8)		2.8 (0.98)	3.6 (2.6)	15 (12)	
62	7.2 (0.69)		11 (2.2)	4.8 (0.82)	26 (10)	
63	1.8 (0.03)	0.15 (0.03)	6.8 (21)	0.16 (0.69)	1.6 (18)	0.69 (0.87)
64	31 (2.1)	69 (2.1)	46 (8.6)	31 (3.4)	49 (9.8)	110 (3.2)
65	36 (3.5)		53 (6.8)	10 (1.5)	55 (21)	22 (9.9)
67	0.10 (<0.05)		<0.05 (<0.05)	<0.05 (<0.05)	0.42 (<0.05)	<0.05 (<0.05)
68	0.14 (<0.05)	1.1 (0.93)	0.11 (0.11)	0.19 (0.07)	0.23 (0.10)	0.08 (<0.05)
69	0.14 (0.60)	3.8 (15)	0.11 (0.24)	0.19 (0.07)	0.23 (0.10)	0.08 (3.2)
71	0.90 (0.36)		0.72 (0.40)	0.92 (0.38)	3.7 (2.6)	4.6 (5.0)

Case No.	Blood (F) (n=20)	Frontal Cortex (n=9)	Bile (n=17)	Vitreous (n=17)	Urine (n=16)	Liver (n=17)
72	1.8 (0.05)		1.1 (0.39)	1.9 (0.71)	2.0 (0.71)	
73	1.8 (0.83)		0.70 (0.39)		8.5 (0.05)	4.8 (1.8)
82	0.19 (0.76)					
Mean ± SD	4.3 ± 9.3 (0.92 ± 1.1)	8.4 ± 21 (4.6 ± 6.0)	7.4 ± 13 (4.8 ± 6.6)	3.3 ± 7.3 (1.0 ± 1.1)	16 ± 18 (23 ± 59)	4.8 ± 8.5 (3.0 ± 3.3)
Range	0.07-36 (<0.05-3.5)	0.40-69 (<0.05-16)	<0.05-53 (<0.05-21)	<0.05-31 (<0.05-3.4)	0.19-55 (<0.05-250)	<0.05-110 (<0.05-9.9)
Median	1.10 (0.64)	1.2 (2.3)	1.3 (1.2)	0.78 (0.79)	8.5 (5.1)	1.8 (1.8)

[1] F = femoral; H = heart. [2] All concentrations in mg/L or mg/kg; metabolite concentrations expressed in parentheses.

5.3.2.2. Venlafaxine tissue:blood concentration ratios

The ratios of venlafaxine concentrations in tissue to femoral blood are shown in Figure 5.3. The

concentration ratio in urine was higher than for any other tissue. The next highest concentration

ratios were observed in bile and frontal cortex, while the ratio in vitreous humour was the lowest.
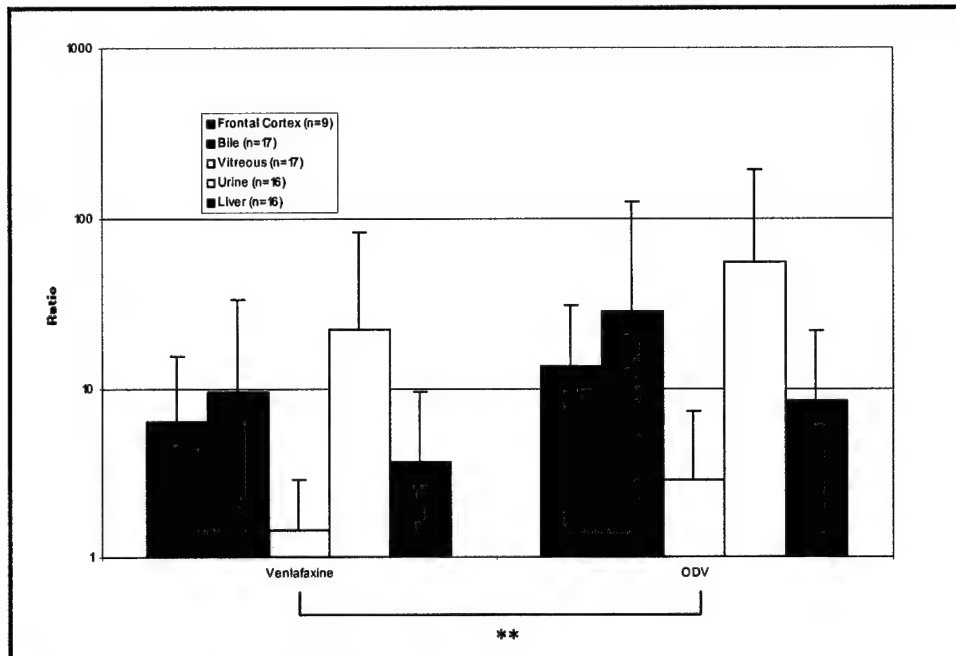
*Figure 5.3. Concentration ratios of venlafaxine and O-desmethylvenlafaxine in postmortem tissues compared with femoral blood (means ±SD's). ** p<0.01.*

5.3.2.3. Correlation of blood venlafaxine and O-desmethylvenlafaxine concentrations to those in other tissues

All tissues showed a significant correlation with blood for venlafaxine (Table 5.4, see also Figures 5.4 and 5.5).

The correlations for O-desmethylvenlafaxine were much lower. The only significant combinations were with liver and vitreous humour.

Table 5.4. Venlafaxine correlation data in postmortem tissues compared to femoral blood[1,2].

Tissue	Pearson Correlation (r^2)	95% confidence interval for m	p-value
Frontal Cortex	**0.949**	**0.357-0.524**	**0.000**
	(0.018)	(-0.167-0.120)	(0.714)
Bile	**0.472**	**0.243-0.864**	**0.000**
	(0.009)	(-0.077-0.110)	(0.715)
Vitreous	**0.674**	**0.744-1.621**	**0.000**
	(0.223)	**(0.005-1.042)**	**(0.048)**
Urine	**0.664**	**0.293-0.670**	**0.000**
	(0.000)	(-0.011-0.011)	(0.976)
Liver	**0.875**	**0.952-1.451**	**0.000**
	(0.354)	**(0.053-0.359)**	**(0.012)**

[1] Significant correlations in boldface. [2] Metabolite correlation data expressed in parentheses.
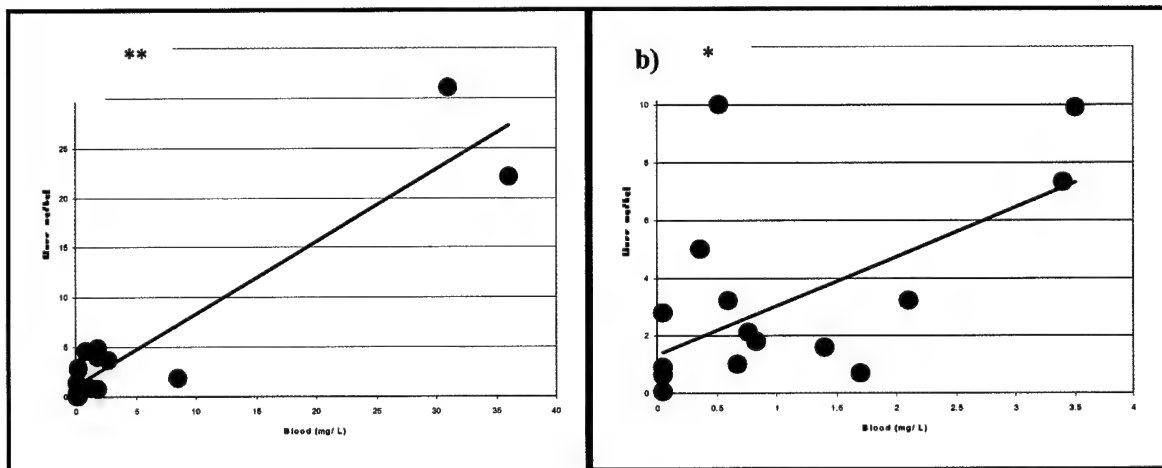
Figure 5.4. Scattergrams showing positive correlation between liver and femoral blood a) venlafaxine concentrations and b) O-desmethylvenlafaxine concentrations. *p<0.05, **p<0.01.
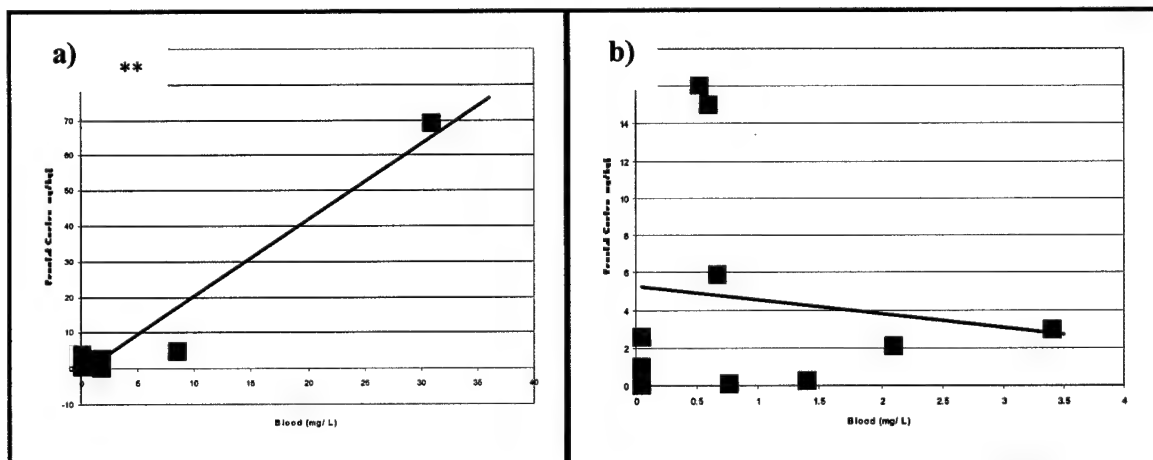
Figure 5.5. Scattergram showing a) positive correlation between frontal cortex and femoral blood venlafaxine concentrations and b) no correlation between frontal cortex and femoral blood O-desmethylvenlafaxine concentrations. **p<0.01.

5.3.3. Paroxetine

5.3.3.1. Tissue concentrations of paroxetine -- summary

Paroxetine was detected in 10 cases included in this dissertation (Table 5.5). Like sertraline, the highest mean paroxetine concentrations were detected in liver. The next highest concentrations were detected in femoral blood, frontal cortex, and bile.

Table 5.5. Summary of paroxetine tissue concentrations[1,2].

Case No.	Blood (F) (n=10)	Frontal Cortex (n=9)	Bile (n=7)	Vitreous (n=10)	Urine (n=8)	Liver (n=10)
11	0.73	1.0		1.4	<0.05	3.1
12	2.7	<0.05	<0.05	<0.05	<0.05	0.42
21	<0.05	0.50	<0.05	0.16	<0.05	0.35
22	0.60	9.7	2.4	0.07	0.18	4.4
23	1.1	1.2		0.07	<0.05	3.1
32	4.8	0.57	0.33	1.2	0.21	0.52
33	11	0.91	0.66	2.0	0.12	4.4
44	<0.05	1.9		<0.05		2.6
45	2.9		4.1	0.34	1.0	22
46	14	2.4	1.6	2.4		13
Mean ± SD	3.8 ± 4.9	2.0 ± 3.0	1.3 ± 1.5	0.77 ± 0.90	0.21 ± 0.32	5.4 ± 6.9
Range	<0.05-14	<0.05-9.7	<0.05-4.1	<0.05-2.4	<0.05-1.0	0.35-22
Median	1.9	1.0	0.66	0.25	0.09	3.1

[1] F = femoral; H = heart. [2] All concentrations in mg/L or mg/kg.

5.3.3.2. Paroxetine tissue:blood concentration ratios

The ratios of the mean paroxetine concentrations in tissue to femoral blood are shown in Figure

5.6. The highest and lowest ratios were observed in liver and urine, respectively.
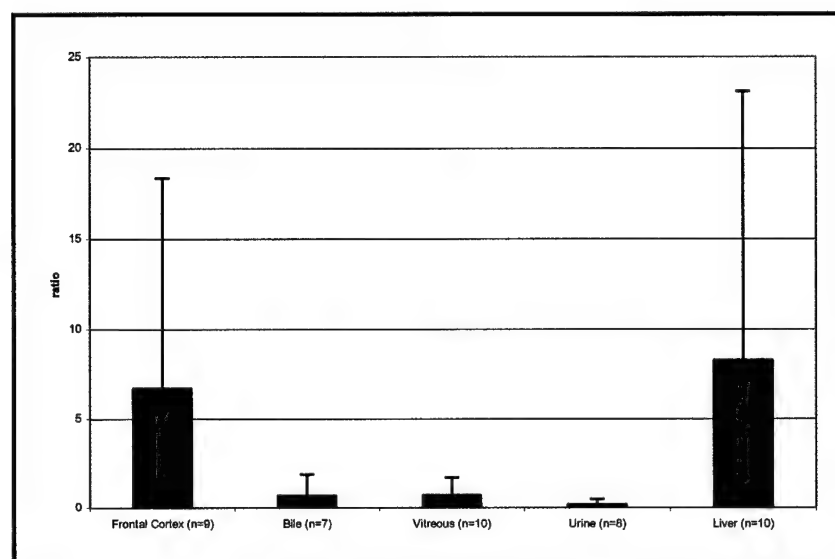
Figure 5.6. Concentration ratios of paroxetine in postmortem tissues compared with femoral

blood (means ± SD's).

5.3.3.3. Correlation of blood paroxetine concentrations to those in other tissues

Vitreous humour showed a significant correlation with blood for paroxetine (Table 5.6, see also

Figure 5.7). None of the correlations for any other tissues were highly positive or significant.

Table 5.6. Paroxetine correlation data of postmortem tissues compared to femoral blood[1].

Tissue	Pearson Correlation (r^2)	95% confidence interval for m	p-value
Vitreous	0.749	2.473-6.885	0.001
Liver	0.113	-0.305-0.781	0.341
Urine	0.003	-10.442-11.694	0.894
Bile	0.003	-4.254-3.869	0.908
Frontal Cortex	0.018	-1.775-1.307	0.730

[1] Significant correlations in boldface.
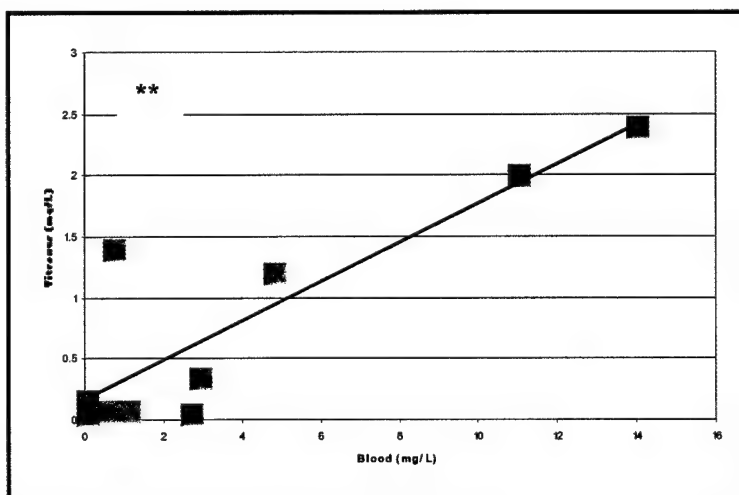
Figure 5.7. Scattergram showing positive correlation between vitreous humour and femoral blood paroxetine concentrations. **p<0.01.

5.3.4. *Fluoxetine*

5.3.4.1. Tissue concentrations of fluoxetine -- summary

There were 10 cases studied in these experiments that tested positive for fluoxetine. Individual data as well as mean, range and median concentrations of both fluoxetine and norfluoxetine in each tissue are shown in Table 5.7. The highest mean concentrations of fluoxetine were detected in liver, followed by frontal cortex, bile, and urine. The lowest mean fluoxetine concentrations were again detected in vitreous humour. On average the norfluoxetine to fluoxetine ratio was 1.5:1, ranging from 0.07-14:1. As opposed to the metabolites for sertraline and venlafaxine, norfluoxetine blood concentrations were not significantly correlated with those of fluoxetine ($r^2 = 0.037$, $p > 0.05$).

5.3.4.2. Fluoxetine tissue:blood concentration ratios

The ratios of the mean concentrations of fluoxetine and norfluoxetine in tissue to femoral blood are shown in Figure 5.8. The concentration ratios of liver and frontal cortex were much higher than for the other tissues. The lowest concentration ratios were observed in vitreous humour.

Table 5.7. Summary of fluoxetine and norfluoxetine tissue concentrations[1,2] .

Case No.	Blood (F) (n=10)	Frontal Cortex (n=9)	Bile (n=9)	Vitreous (n=10)	Urine (n=7)	Liver (n=10)
1	<0.05 (0.12)	8.9 (2.5)	1.2 (0.50)	0.16 (0.19)	0.67 (0.38)	9.2 (6.0)
2	0.09 (0.18)	0.37 (0.25)	<0.05 (0.11)	0.16 (0.19)	0.09 (0.08)	<0.05 (<0.05)
3	0.30 (<0.05)	3.1 (3.6)	7.6 (10)	<0.05 (<0.05)	0.10 (0.31)	3.9 (4.0)
19	0.87 (0.39)	9.1 (4.1)	9.8 (4.1)	0.26 (0.20)		16 (3.8)
16	0.07 (0.49)	0.42 (0.24)		0.16 (0.18)		0.09 (<0.05)
17	0.08 (0.08)	0.44 (0.28)	0.12 (0.23)	0.15 (0.18)	0.30 (0.08)	0.08 (1.1)
18	0.76 (0.16)		7.7 (3.9)	0.16 (0.18)	0.13 (0.06)	12 (4.7)
30	0.29 (0.18)	4.1 (0.29)	31 (2.8)	0.29 (0.18)	5.9 (0.1)	<0.05 (<0.05)
31	0.40 (0.80)	6.4 (33)	4.9 (11)	0.24 (0.50)	1.3 (1.7)	2.2 (6.0)
41	0.39 (0.22)	24 (14)	5.2 (12)	0.11 (0.24)	0.34 (<0.05)	35 (49)
Mean ± SD	0.30±0.34 (0.31±0.24)	7.1±8.4 (7.7±12)	4.1±3.8 (4.6±5.0)	0.18±0.05 (0.23±0.11)	0.47±0.45 (0.39±0.65)	9.3±12 (8.8±16)
Range	<0.05-0.87 (<0.05-0.80)	0.37-24 (0.24-33)	<0.05-31 (0.11-12)	<0.05-0.29 (<0.05-0.50)	0.09-5.9 (<0.05-1.7)	<0.05-35 (<0.05-49)
Median	0.30 (0.18)	4.1 (2.5)	4.9 (3.9)	0.16 (0.19)	0.32 (0.09)	3.1 (3.9)

[1] F = femoral; H = heart. [2] All concentrations in mg/L or mg/kg; metabolite concentrations expressed in parentheses.
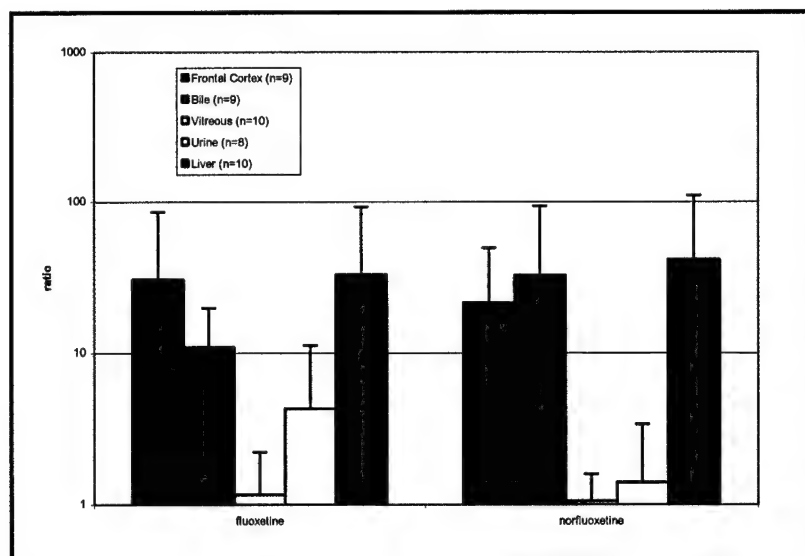
Figure 5.8. Concentration ratios of fluoxetine and norfluoxetine in postmortem tissues compared with femoral blood (means ± SD's).

5.3.4.3. Correlation of blood fluoxetine and norfluoxetine concentrations to those in other tissues

Bile concentrations exhibited the only significant correlation with blood for fluoxetine (Table 5.8, see also Figure 5.9). By contrast, urine, vitreous humour, and frontal cortex concentrations of norfluoxetine all showed statistically significant correlations with blood (Figure 5.10).

Table 5.8. Fluoxetine correlation data of postmortem tissues compared to femoral blood[1,2].

Tissue	Pearson Correlation (r^2)	95% confidence interval for m	p-value
Bile	**0.787**	**0.039-0.125**	**0.003**
	(0.426)	(-0.005-0.070)	(0.079)
Liver	0.263	-0.007-0.035	0.158
	(0.004)	(-0.014-0.012)	(0.865)
Frontal Cortex	0.177	-0.193-0.461	0.299
	(0.572)	**(0.002-0.030)**	**(0.030)**
Vitreous	0.051	-3.416-5.819	0.558
	(0.666)	**(0.667-2.959)**	**(0.007)**
Urine	0.000	-0.147-0.142	0.969
	(0.905)	**(0.243-0.533)**	**(0.001)**

[1] Significant correlations in boldface. [2] Metabolite correlation data expressed in parentheses.
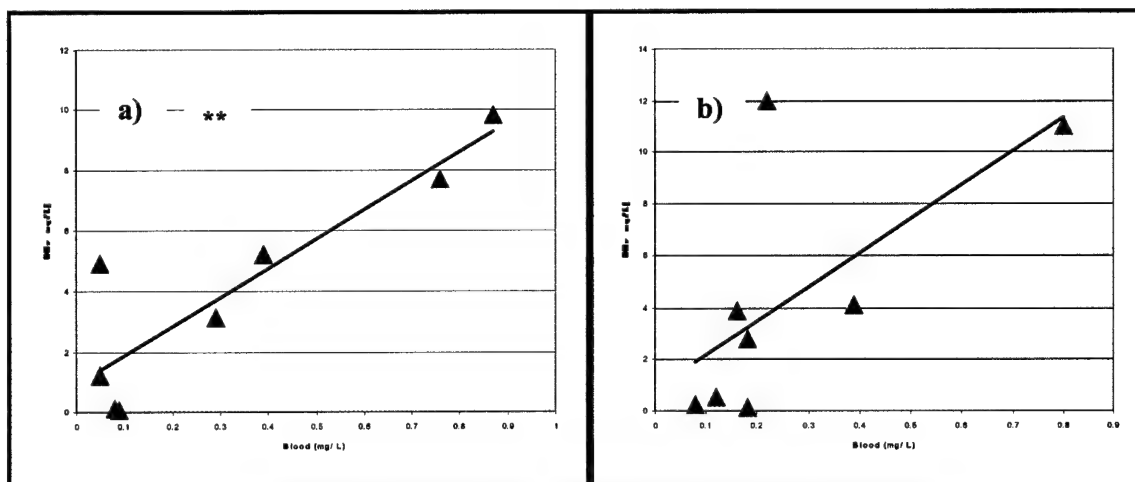
Figure 5.9. Scattergrams showing correlation between bile and femoral blood for a) fluoxetine and b) norfluoxetine. ** *p<0.01.*
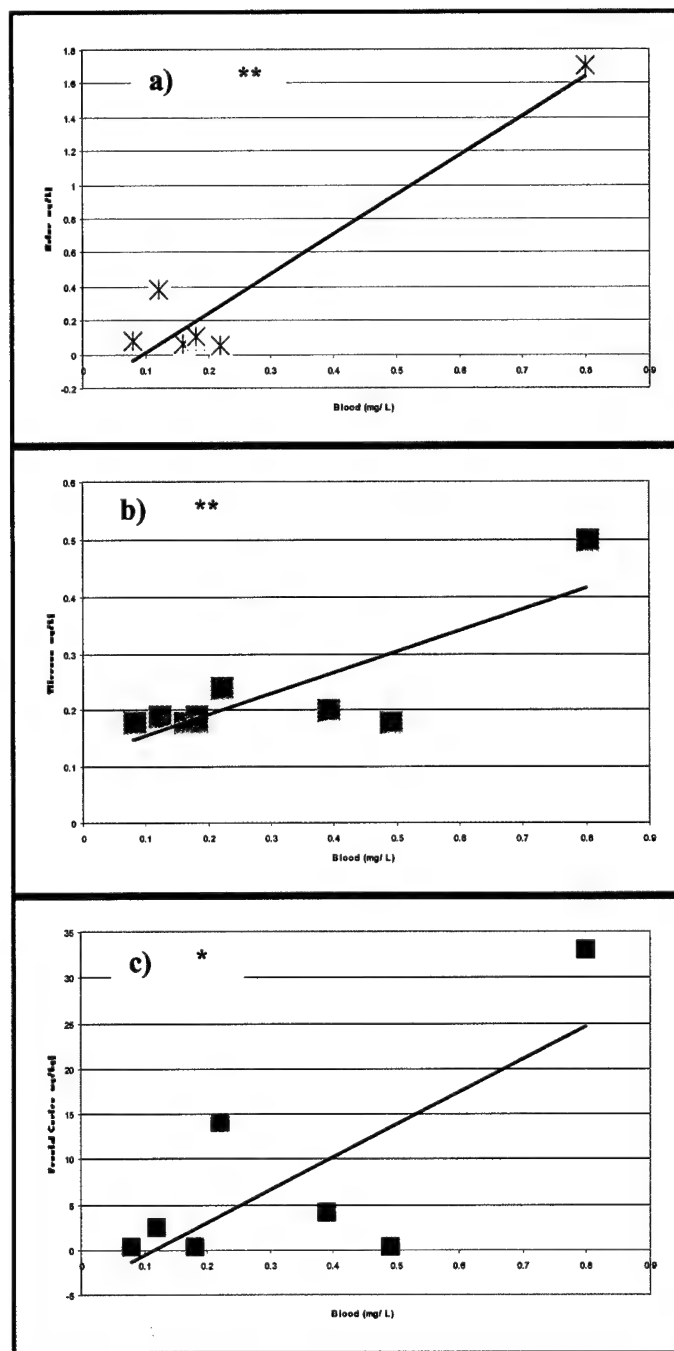
Figure 5.10. Scattergrams showing norfluoxetine correlations between femoral blood and a) urine, b) vitreous humour, and c) frontal cortex. $^*p<0.05,$ $^{**}p<0.01.$

5.3.5. Risperidone

5.3.5.1. Tissue concentrations of risperidone -- summary

The mean, range, and median tissue concentrations of risperidone measured in 9 cases are shown in Table 5.9. The highest mean concentration of risperidone was detected in bile, followed by frontal cortex, liver, and urine. The lowest mean concentrations were detected in femoral blood and vitreous humour. The ratio of 9-OH-risperidone to risperidone averaged 5.9:1; however, it ranged from 0.04-100:1. Blood concentrations of 9-OH-risperidone showed only a slightly positive correlation with those of risperidone, and this correlation was not significant ($r^2 = 0.211$, $p > 0.05$).

5.3.5.2. Risperidone tissue:blood concentration ratios

The ratios of the mean concentrations of risperidone in tissue to femoral blood are shown in Figure 5.11. The highest concentration ratios were observed in bile and frontal cortex, while the vitreous humour concentration ratio was the lowest.

Table 5.9. Summary of risperidone and 9-OH-risperidone tissue concentrations[1,2].

Case No.	Blood (F) (n=9)	Frontal Cortex (n=8)	Bile (n=8)	Vitreous (n=8)	Urine (n=6)	Liver (n=8)
3	0.30 (0.40)	0.24 (0.18)	1.1 (0.04)	0.02 (0.03)	<0.02 (<0.02)	0.16 (0.27)
6	0.22 (0.25)	0.08 (0.05)	2.0 (0.69)	0.51 (0.73)	1.0 (1.9)	0.22 (<0.02)
74	0.03 (<0.02)	<0.02 (0.17)	0.23 (0.07)	<0.02 (0.05)		<0.02 (<0.02)
75	0.07 (0.26)	0.68 (0.25)	0.06 (6.2)	0.04 (0.09)	0.09 (2.9)	0.61 (0.18)
77	<0.02 (<0.02)					
78	0.36 (0.09)	1.3 (0.36)	9.3 (11)	0.43 (0.05)		0.98 (0.10)
79	0.09 (0.02)	1.0 (0.54)	1.1 (0.25)	0.03 (0.03)	0.08 (0.05)	1.0 (0.04)
80	0.12 (0.12)	0.51 (2.0)	1.1 (0.89)	0.18 (0.19)	<0.02 (1.5)	0.50 (1.3)
81	0.13 (0.29)	0.78 (0.89)	0.17 (1.9)	0.05 (0.45)	0.49 (3.0)	0.10 (0.15)
Mean ± SD	0.15 ± 0.12 (0.16 ± 0.14)	0.58 ± 0.45 (0.56 ± 0.64)	1.9 ± 3.1 (2.6 ± 4.0)	0.16 ± 0.20 (0.20 ± 0.26)	0.28 ± 0.39 (1.6 ± 1.3)	0.45 ± 0.39 (0.26 ± 0.43)
Range	<0.02-0.36 (<0.02-0.40)	<0.02-1.3 (<0.02-0.89)	0.06-9.3 (0.04-11)	<0.02-0.51 (0.03-0.73)	<0.02-1.0 (<0.02-3.0)	<0.02-0.98 (<0.02-1.3)
Median	0.12 (0.12)	0.60 (0.31)	1.1 (0.79)	0.05 (0.07)	0.09 (1.7)	0.36 (0.13)

[1] F = femoral; H = heart. [2] All concentrations in mg/L or mg/kg; metabolite concentrations expressed in parentheses.
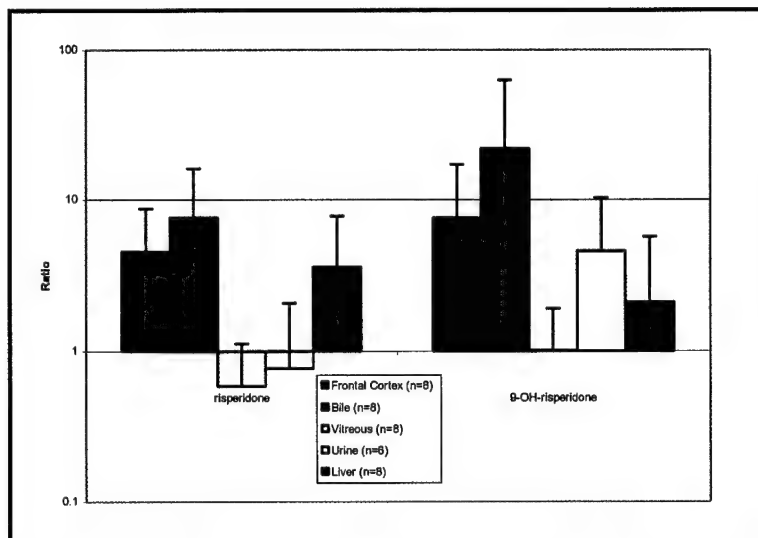
Figure 5.11. Concentration ratios of risperidone and 9-OH-risperidone in postmortem tissues compared with femoral blood (means ± SD's).

5.3.5.3. Correlation of blood risperidone and 9-hydroxyrisperidone concentrations to those in other tissues

Bile risperidone concentrations exhibited the only statistically significant correlation with blood, and no significant correlations or trends with blood were observed for 9-hydroxyrisperidone (Table 5.10, see also Figure 5.12). The correlations with blood for risperidone were higher in each tissue than those for 9-OH-risperidone.

Table 5.10. Risperidone correlation data of postmortem tissues compared to femoral blood[1,2].

Tissue	Pearson Correlation (r^2)	95% confidence interval for m	p-value
Bile	**0.573**	**0.004-0.054**	**0.030**
	(0.009)	(-0.038-0.032)	(0.825)
Vitreous	0.347	-0.128-0.817	0.124
	(0.113)	(-0.327-0.692)	(0.415)
Urine	0.059	-0.246-0.355	0.642
	(0.049)	(-0.115-0.161)	(0.673)
Frontal Cortex	0.070	-0.180-0.317	0.526
	(0.032)	(-0.251-0.174)	(0.674)
Liver	0.040	-0.234-0.354	0.637
	(0.000)	(-0.329-0.317)	(0.966)

[1] Significant correlations in boldface. [2] Metabolite correlation data expressed in parentheses.
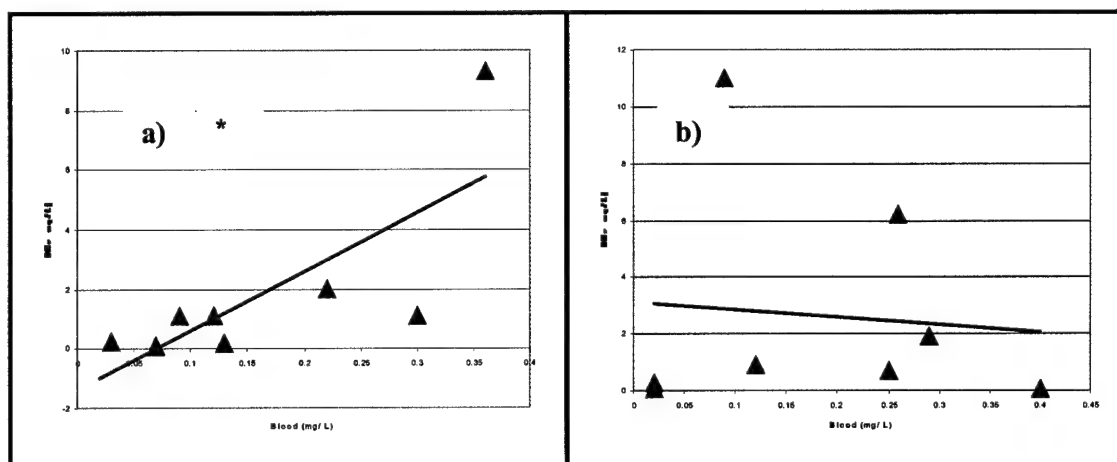
Figure 5.12. Scattergrams showing a) correlation between bile and femoral blood risperidone concentrations and b) lack of correlation between bile and femoral blood 9-hydroxyrisperidone concentrations. * p<0.05.

5.3.6. Citalopram

5.3.6.1. Tissue concentrations of citalopram -- summary

Citalopram was present in 7 cases included in these experiments. Individual data, as well as mean, range, and median tissue concentrations for these cases are shown in Table 5.11. The highest mean citalopram concentrations were again detected in liver, followed by frontal cortex, bile, and urine. The lowest mean citalopram concentrations were in femoral blood.

5.3.6.2. Citalopram tissue:blood concentration ratios

The ratios of the mean concentrations of citalopram in tissue to femoral blood are shown in Figure 5.13. Like most other drugs already discussed, the highest concentration ratios were observed in liver and the lowest in vitreous humour.

Table 5.11. Summary of citalopram tissue concentrations[1,2].

Case No.	Blood (F) (n=7)	Frontal Cortex (n=6)	Bile (n=5)	Vitreous (n=6)	Urine (n=5)	Liver (n=6)
9	0.14	0.52	3.2	0.21	0.53	1.8
10	0.15	0.10	1.8	<0.05	1.3	0.21
14	0.39	5.0		0.28	1.4	20
15	1.9	12	6	2.3	23	6.1
29	1.6					
39	0.10	<0.05	0.56	0.21	3.9	11
40		22	27	5.0		45
Mean ± SD	0.71 ± 0.82	6.6 ± 8.8	7.7 ± 11	1.3 ± 2.0	6.0 ± 9.6	14 ± 17
Range	0.14-1.9	<0.05-22	0.56-27	<0.05-5.0	0.53-23	0.21-44
Median	0.27	2.8	3.2	0.25	1.4	8.6

[1] F = femoral; H = heart.   [2] All concentrations in mg/L or mg/kg.
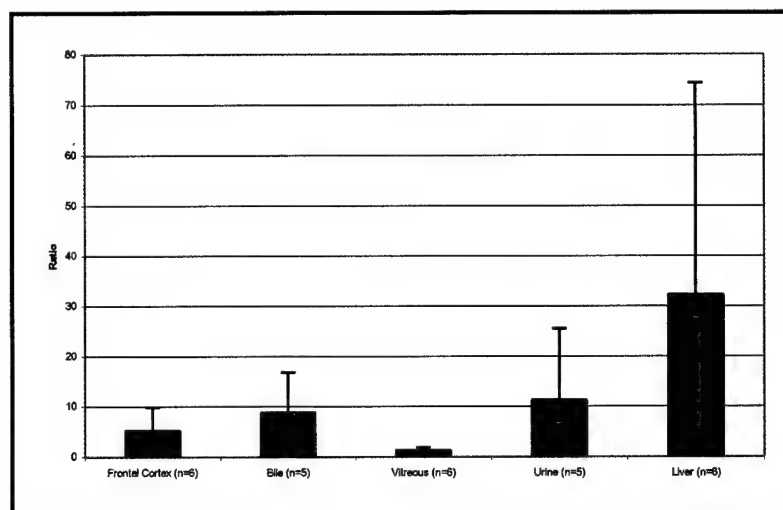
Figure 5.13. Concentration ratios of citalopram in postmortem tissues compared with femoral blood (means ± SD's).

5.3.6.3. Correlation of blood citalopram concentrations to those in other tissues

Citalopram concentrations in all tissues except liver exhibited significant correlations with blood (Table 5.12, see also Figures 5.14 and 5.15). Although not statistically significant, the correlations of liver citalopram concentrations to blood were also moderately positive.

Table 5.12. Citalopram correlation data of postmortem tissues compared to femoral blood[1].

Tissue	Pearson Correlation (r^2)	95% confidence interval for m	p-value
Vitreous	0.977	0.491-0.759	0.001
Frontal Cortex	0.979	0.111-0.169	0.001
Urine	0.949	0.045-0.112	0.005
Bile	0.821	0.016-0.210	0.034
Liver	0.568	-0.012-0.124	0.084

[1] Signicant correlations in boldface.
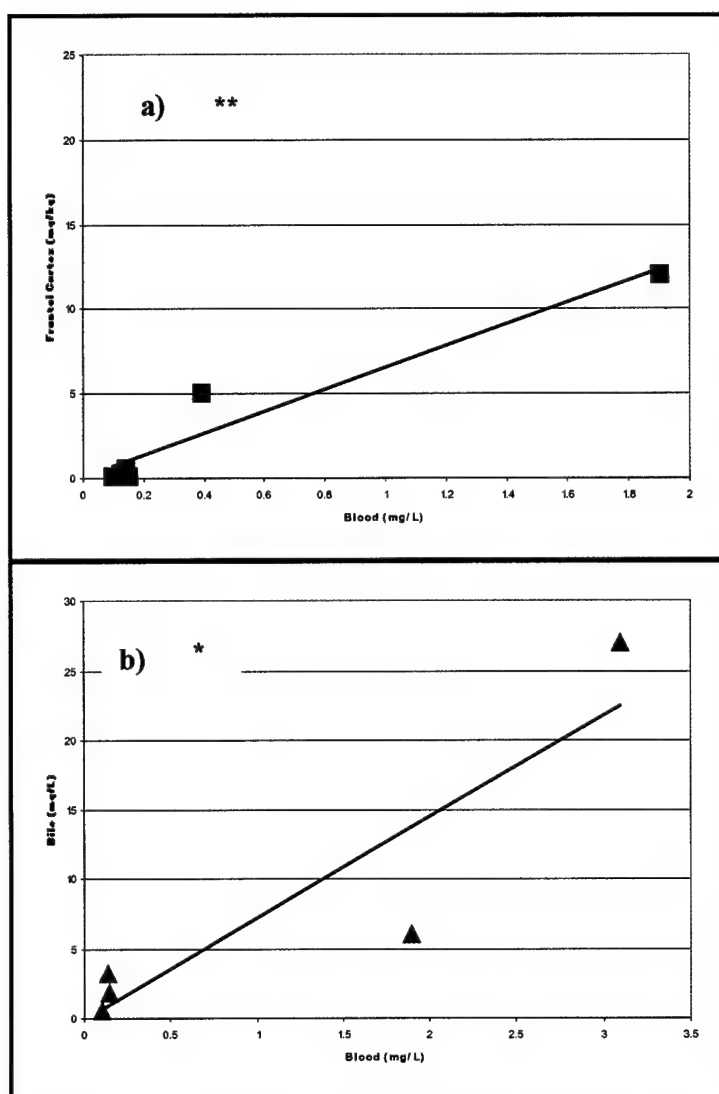
Figure 5.14. Scattergrams showing correlation between a) frontal cortex and femoral blood and b) bile and femoral blood. $^*p<0.05$, $^{**}p<0.01$.
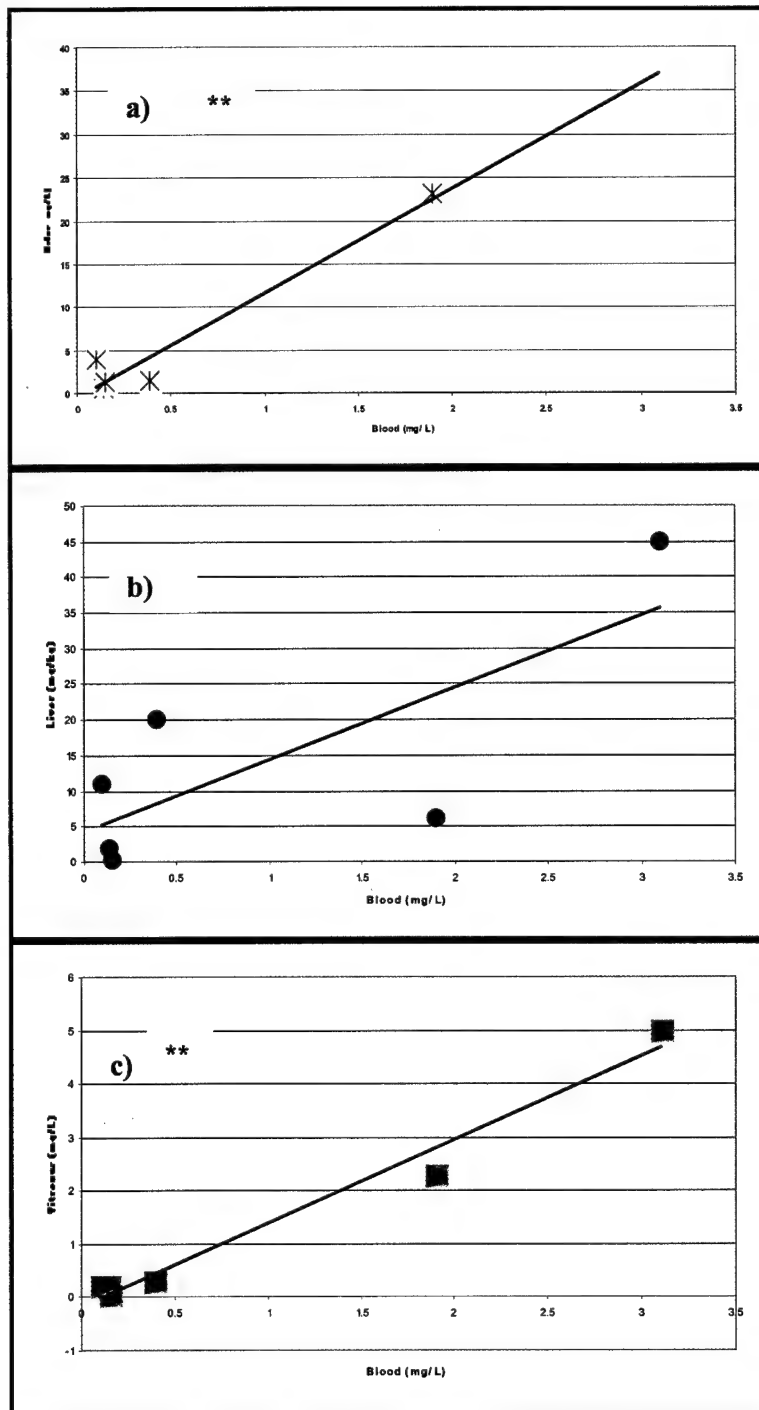
*Figure 5.15. Scattergrams showing correlation between a) urine and femoral blood, b) liver and femoral blood and c) vitreous humour and femoral blood. ** p<0.01.*

5.3.7. *Nefazodone*

5.3.7.1. Tissue concentrations of nefazodone -- summary

Nefazodone was detected in 6 cases. Tissue nefazodone concentrations detected in these cases are shown in Table 5.13. Mean nefazodone concentrations were highest in bile, followed by liver, frontal cortex, and femoral blood. As with several other drugs already discussed, nefazodone was detected at lowest mean concentrations in vitreous humour.

5.3.7.2. Nefazodone tissue:blood concentration ratios

The ratios of the mean concentrations of nefazodone in tissue to femoral blood are shown in Figure 5.16. The highest concentration ratios were observed with liver and urine, while the vitreous humour concentration ratio was the lowest.

Table 5.13. Summary of nefazodone tissue concentrations[1,2].

Case No.	Blood (F) (n=6)	Frontal Cortex (n=5)	Bile (n=4)	Vitreous (n=6)	Urine (n=5)	Liver (n=5)
30	2.8	34	22	0.10	0.16	40
41	0.13	0.21	0.10	0.11	4.9	
51	1.1	0.53		0.10		3.0
54	4.7		39	0.99	0.11	15
58	0.30	1.7	<0.05	<0.05	<0.05	2.4
66	0.23	<0.05		0.10	0.32	7.0
Mean ± SD	1.5 ± 1.8	9.1 ± 17	20 ± 20	0.24 ± 0.40	1.4 ± 2.4	13 ± 16
Range	0.13-4.7	<0.05-34	<0.05-39	<0.05-0.99	<0.05-4.9	2.4-40
Median	0.70	0.53	11	0.10	0.16	7.0

[1] F = femoral; H = heart.   [2] All concentrations in mg/L or mg/kg.
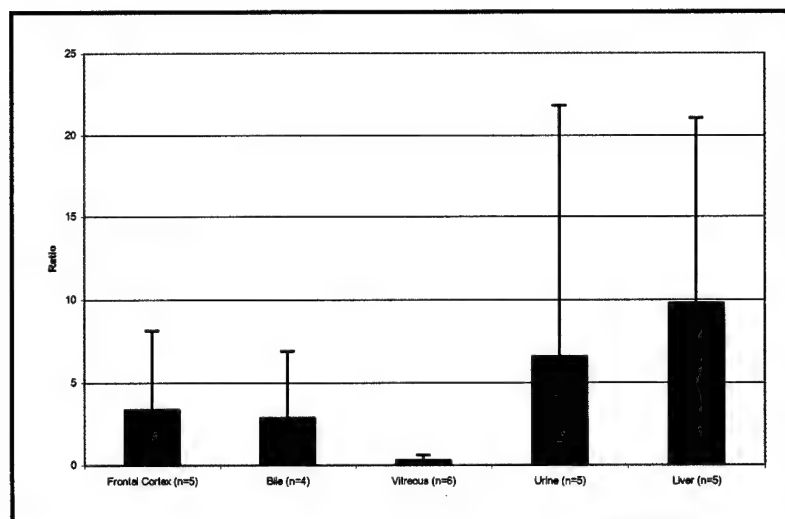
Figure 5.16. Concentration ratios of nefazodone in postmortem tissues compared with femoral blood (means ±SD's).

5.3.7.3. Correlation of blood nefazodone concentrations to those in other tissues

Bile, frontal cortex, and vitreous humour all exhibited highly positive, statistically significant correlations with blood for nefazodone (Table 5.14, see also Figure 5.17). Liver and urine concentrations were not significantly correlated with blood (Figure 5.18).
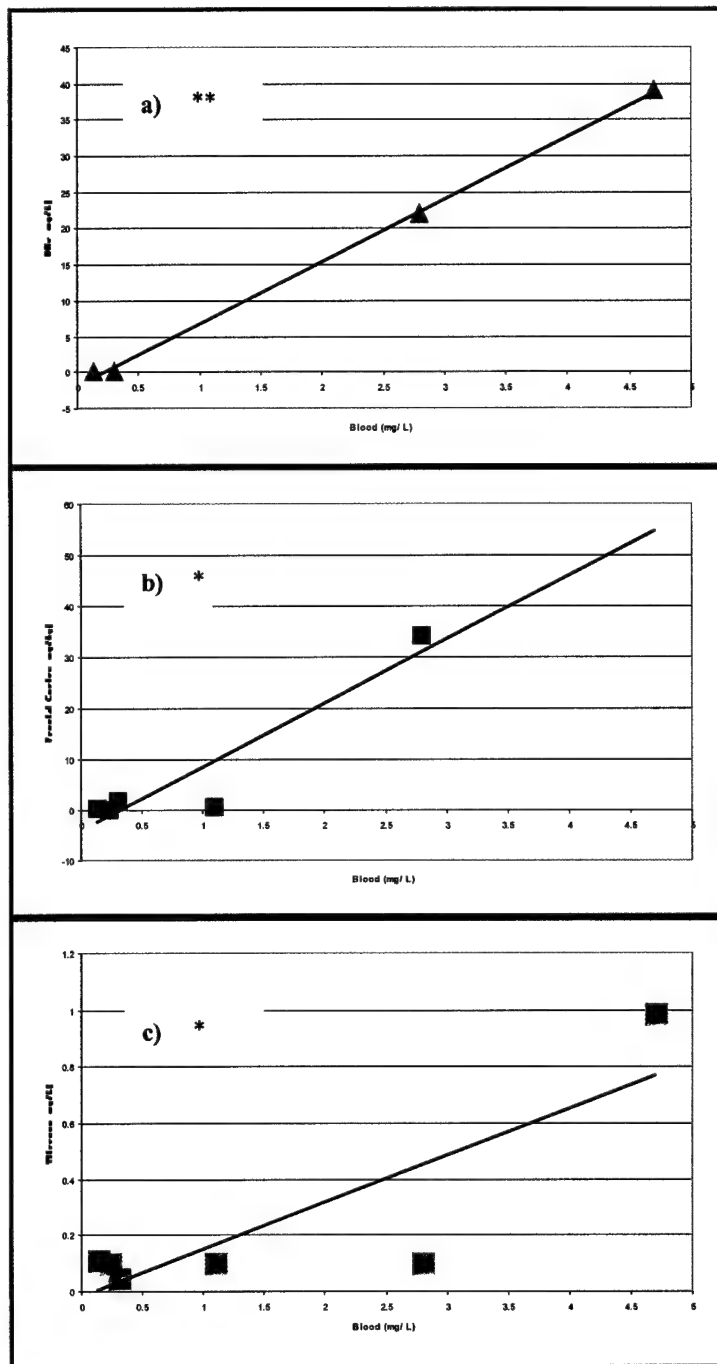
Figure 5.17. Scattergrams showing correlations between a) bile and femoral blood, b) frontal cortex and femoral blood and c) vitreous humour and femoral blood. $^*p<0.05$, $^{**}p<0.01$.
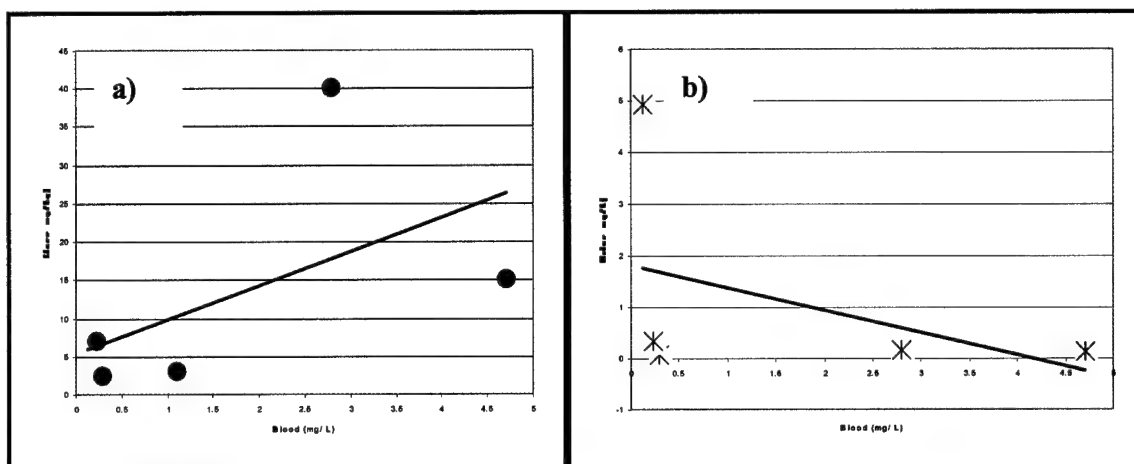
Figure 5.18. Scattergrams showing lack of correlation between a) liver and femoral blood and b) urine and femoral blood.

Table 5.14. Nefazodone correlation data of postmortem tissues compared to femoral blood[1].

Tissue	Pearson Correlation (r^2)	95% confidence interval for m	p-value
Bile	**0.999**	**0.103-0.128**	**0.001**
Frontal Cortex	**0.881**	**0.023-0.118**	**0.018**
Vitreous	**0.712**	**0.496-7.980**	**0.035**
Liver	0.296	-0.122-0.255	0.344
Urine	0.179	-2.015-1.199	0.478

[1] Significant correlations in boldface.

5.3.8. Fluvoxamine

5.3.8.1. Tissue concentrations of fluvoxamine -- summary

Fluvoxamine was detected in 2 cases. Tissue concentrations detected in these cases are shown in Table 5.15. The highest mean fluvoxamine concentrations were detected in liver, followed by

frontal cortex, urine, and bile. The lowest mean fluvoxamine concentrations were again detected in vitreous humour.

5.3.8.2. Fluvoxamine tissue:blood concentration ratios

The ratios of the mean concentrations of fluvoxamine in tissue to femoral blood are shown in Figure 5.19. The highest concentration ratios were observed in liver, followed by both frontal cortex and urine, with approximately equal concentration ratios. The lowest ratios were observed in vitreous humour.
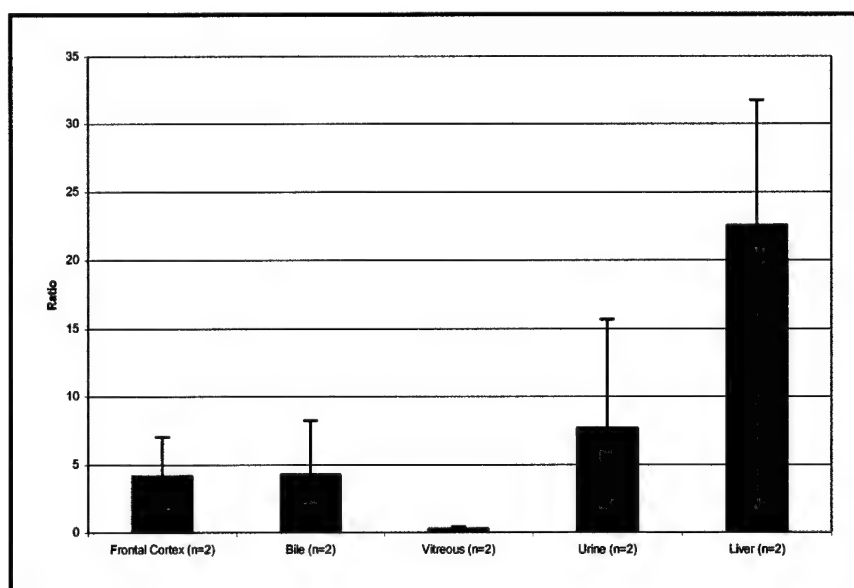
Figure 5.19. Concentration ratios of fluvoxamine in postmortem tissues compared with femoral blood (means ±SD's).

Table 5.15. Summary of fluvoxamine tissue concentrations[1,2].

Case No.	Blood (F) (n=2)	Frontal Cortex (n=2)	Bile (n=2)	Vitreous (n=2)	Urine (n=2)	Liver (n=2)
20	0.75	1.6	5.3	0.29	10	12
43	2.1	13	3.1	0.34	4.2	61
Mean ±SD	1.4 ± 0.95	7.3 ± 8.0	4.2 ±1.6	0.32 ± 0.04	7.1 ± 4.1	37 ± 35
Range	0.75-2.0	1.6-13	3.1-5.3	0.29-0.34	4.2-10	12-61
Median	1.4	7.3	4.2	0.32	7.1	37

[1] F = femoral; H = heart. [2] All concentrations in mg/L or mg/kg.

There were too few fluvoxamine-positive cases to perform a correlation between tissue and femoral blood concentrations. This test was therefore not performed.

5.4. Discussion

As expected, each drug was unevenly distributed throughout the tissues analysed, although their patterns of distribution did follow certain trends. Table 5.16 summarises tissue concentrations for each drug. For citalopram, paroxetine, and risperidone, vitreous humour concentrations were the lowest of all tissues. The highest drug concentrations were found in liver and bile.

Since each of the studied drugs is metabolised in the liver, it is not surprising to detect high concentrations in this tissue. However, distribution into other tissues followed four distinct patterns (see Table 5.17 and Figure 5.20). Pattern A (urine>frontal cortex/bile>femoral blood>vitreous humour) was exhibited by fluvoxamine and venlafaxine. Pattern B (liver>frontal cortex/bile>urine/femoral blood>vitreous fluid) was observed for sertraline, citalopram, and fluoxetine. Paroxetine followed pattern C (liver>femoral blood>frontal cortex>bile>vitreous humour>urine). Finally, nefazodone and risperidone both exhibited pattern D (bile>frontal cortex/liver>urine/femoral blood>vitreous humour).

Table 5.16. Summary of tissue concentrations of selected psychiatric drugs[1,2,3].

Drug	Blood (F)	Frontal Cortex	Bile	Vitreous Fluid	Urine	Liver
Citalopram (n=7)	0.54 ± 0.77	7.9 ± 9.2	7.7 ± 11	1.6 ± 2.1	6.0 ± 10	14 ± 17
Fluoxetine (n=10)	0.30 ± 0.34	7.1 ± 8.4	4.1 ± 3.8	0.18 ± 0.05	0.47 ± 0.45	9.3 ± 12
	(0.31 ± 0.24)	(7.7 ± 12)	(4.6 ± 5.0)	(0.23 ± 0.11)	(0.39 ± 0.65)	(8.8 ± 16)
Fluvoxamine (n=2)	1.4 ± 0.95	7.3 ± 8.0	4.2 ± 1.6	0.32 ± 0.04	7.1 ± 4.1	37 ± 35
Nefazodone (n=6)	1.5 ± 1.8	9.1 ± 17	20 ± 20	0.24 ± 0.40	1.4 ± 2.4	13 ± 16
Paroxetine (n=10)	3.8 ± 4.9	2.0 ± 3.0	1.3 ± 1.5	0.77 ± 0.90	0.21 ± 0.32	5.4 ± 6.9
Risperidone (n=9)	0.15 ± 0.12	0.58 ± 0.45	1.9 ± 3.1	0.16 ± 0.20	0.28 ± 0.39	0.45 ± 0.39
	(0.16 ± 0.14)	(0.56 ± 0.64)	(2.6 ± 4.0)	(0.20 ± 0.26)	(1.6 ± 1.3)	(0.26 ± 0.43)
Sertraline (n=21)	2.0 ± 6.5	2.5 ± 4.0	3.7 ± 5.6	0.11 ± 0.05	0.54 ± 0.74	13 ± 27
	(0.75 ± 1.3)	(14 ± 25)	(24 ± 32)	(0.31 ± 0.36)	(0.75 ± 1.1)	(20 ± 27)
Venlafaxine (n=20)	4.3 ± 9.3	8.4 ± 21	7.4 ± 13	3.3 ± 7.3	16 ± 18	4.8 ± 8.5
	(0.92 ± 1.1)	(4.6 ± 6.0)	(4.8 ± 6.6)	(1.0 ± 1.1)	(23 ± 59)	(3.0 ± 3.3)

[1] F=femoral; H=heart. [2] All concentrations in mg/L or mg/kg; metabolite concentrations expressed in parentheses. [3] Means ± SD's.

Table 5.17. Tissue distribution patterns, corresponding molecular weights and pharmacokinetic parameters of psychiatric drugs[1-3].

Pattern	M.W.	pK_a	logP	V_D	F_b	Relative Sites of Concentration (Highest to Lowest)
A	298 ± 29	9.15 ± 0.13	3.00 ± 0.12	16.5 ± 12.0	0.52 ± 0.35	urine>frontal cortex/bile>blood (f)>vitreous
B	313 ± 9.7	9.50 ± 0.25	4.61 ± 0.89	21.8 ± 6.37	0.91 ± 0.10	liver>frontal cortex/bile>urine/blood (f)/vitreous
C	329	9.83	3.63	16	0.95	liver>blood (f)>frontal cortex>bile>vitreous>urine
D	440 ± 42	9.05 ± 0.21	3.98 ± 0.92	0.87 ± 0.46	0.95 ± 0.06	bile>frontal cortex/liver>urine/ blood (f)/vitreous

[1] means ± SD's of parameters for drugs exhibiting each pattern. [2] pK_a and logP values calculated using CompuDrug software (CompuDrug Inc., 1999).
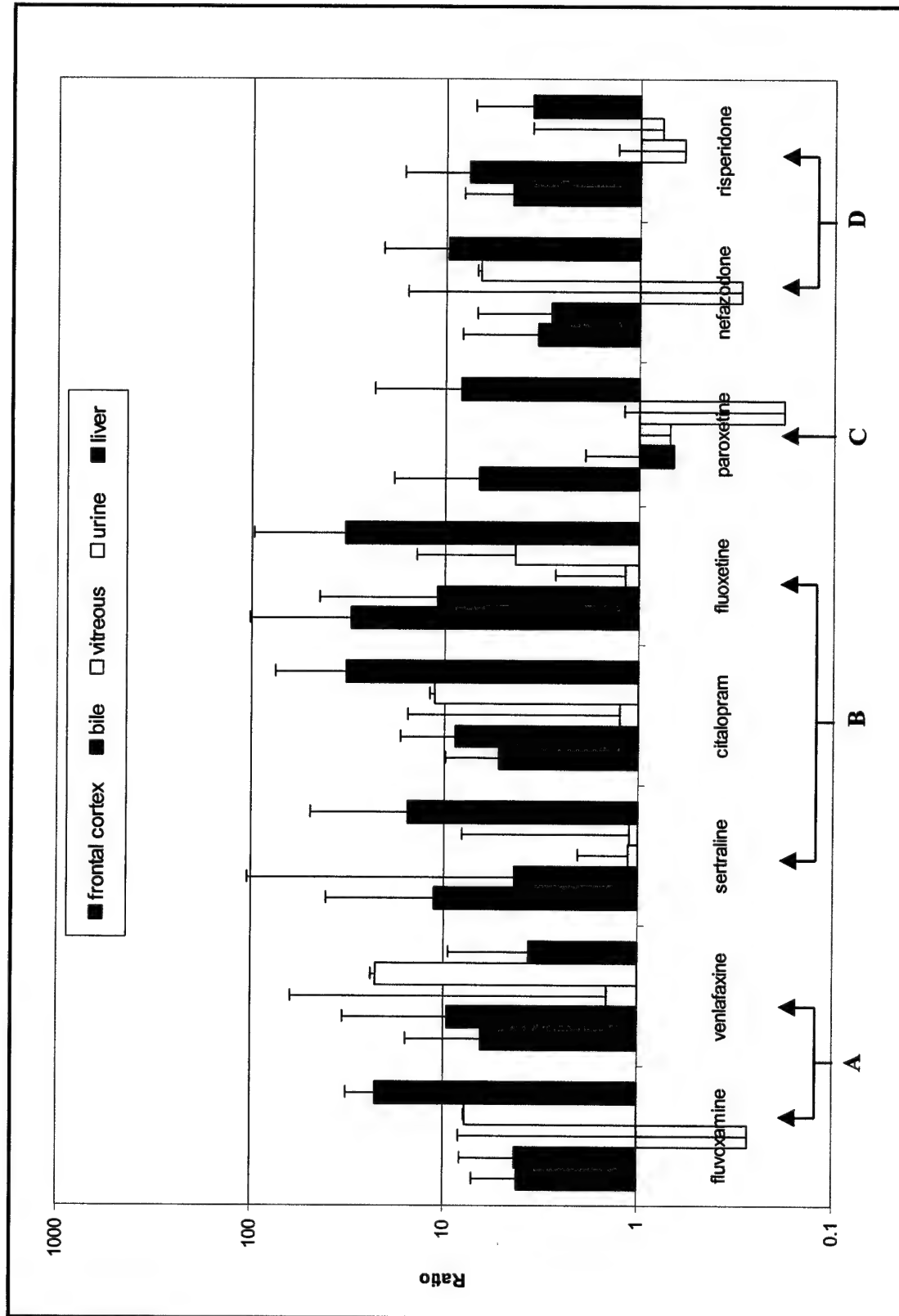
Figure 5.20. Concentration ratios of target psychiatric drugs in tissues compared with femoral blood (means ±SD's).

The drugs exhibiting distribution pattern A were also the most hydrophilic, with logP=3.08 and 2.91 and MW=318 and 277 for fluvoxamine and venlafaxine, respectively (CompuDrug Inc., 1999). Risperidone is also relatively hydrophilic, with logP = 3.33. This may partially explain the relatively higher urine concentrations detected for these drugs since the extent to which a drug is excreted in the urine depends largely on its polarity. This may also explain the lower urine concentrations detected for more lipophilic group B drugs (logP = 3.68, 4.71, and 5.45 for citalopram, fluoxetine, and sertraline, respectively) (CompuDrug Inc., 1999). The presence of pattern A drugs at slightly higher concentrations in urine relative to the other tissues may also stem from their comparatively lower affinity for drug binding proteins. This is because only unbound drug is transported into the extravascular sites in the body (Fb=0.77 and 0.27 for fluvoxamine and venlafaxine, respectively) (Gloor, 1970). However, urine concentrations are difficult to interpret due to variability in volume produced, hydration, and other physiological factors.

It is interesting to note that relative to drug concentrations in other tissues and with the exception of paroxetine, those drugs with higher molecular weight were more likely to be found in bile. The drugs exhibiting tissue distribution pattern A had the lowest mean molecular weight (Table 5.17) and were detected at the lowest relative concentrations in bile out of all groups except group C (paroxetine). There was a slight positive correlation between molecular weight and the bile/blood ratio (r^2 = 0.299) (Figure 5.21). However, this correlation was not statistically significant (p>0.05). This is consistent with the literature. Drugs with a high molecular weight often exhibit a high degree of biliary excretion (Agarwal and Lemos, 1996). Studies on the metabolism and excretion of nefazodone in humans (MW: 470) and risperidone in rats and dogs

(MW: 410 amu) both support this theory, in that they showed evidence of extensive biliary

excretion of these drugs (Barbhaiya et al, 1996; Meuldermans et al, 1994).
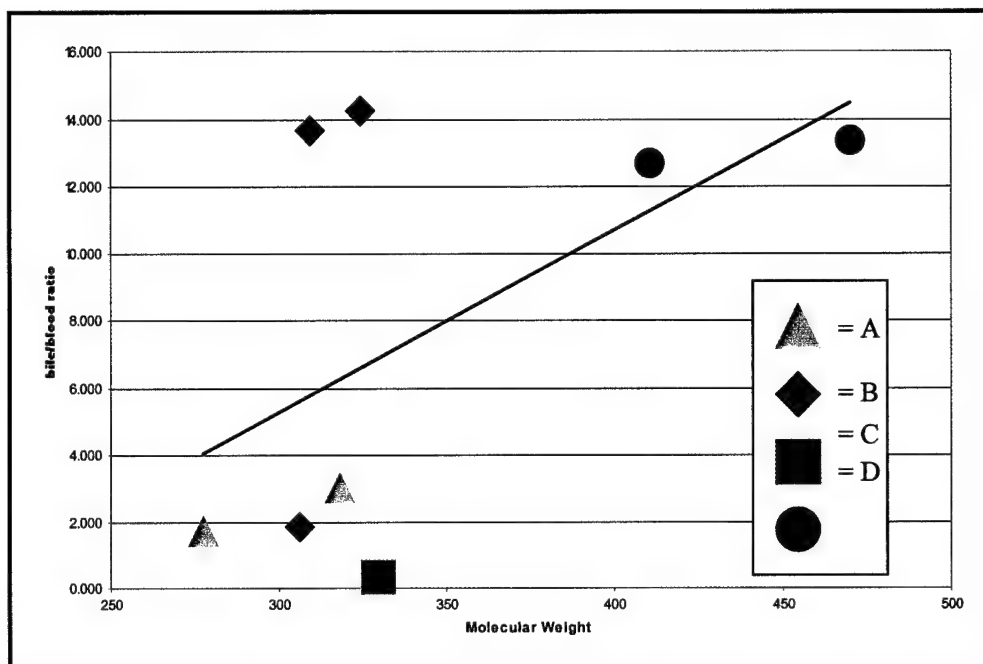
Figure 5.21. Scattergram showing correlation between molecular weight and mean bile/blood concentration ratios with data points labelled according to corresponding distribution pattern.

There was essentially no correlation between lipophilicity and molecular weight of the target

drugs. Molecular weight exhibited a slight, non-significant positive correlation with protein

binding and a significant, negative correlation with volume of distribution ($r^2 = 0.504$, p<0.05)

(Figure 5.22). If compared to tissue:blood concentration ratios, volume of distribution displayed

a slight negative correlation with bile:blood ratios ($r^2 = 0.132$) and a slightly higher correlation

with liver:blood ratios ($r^2 = 0.333$) . However, neither correlation was significant, and no

correlation was observed between volume of distribution and any other tissue:blood

concentration ratios (p>0.05).
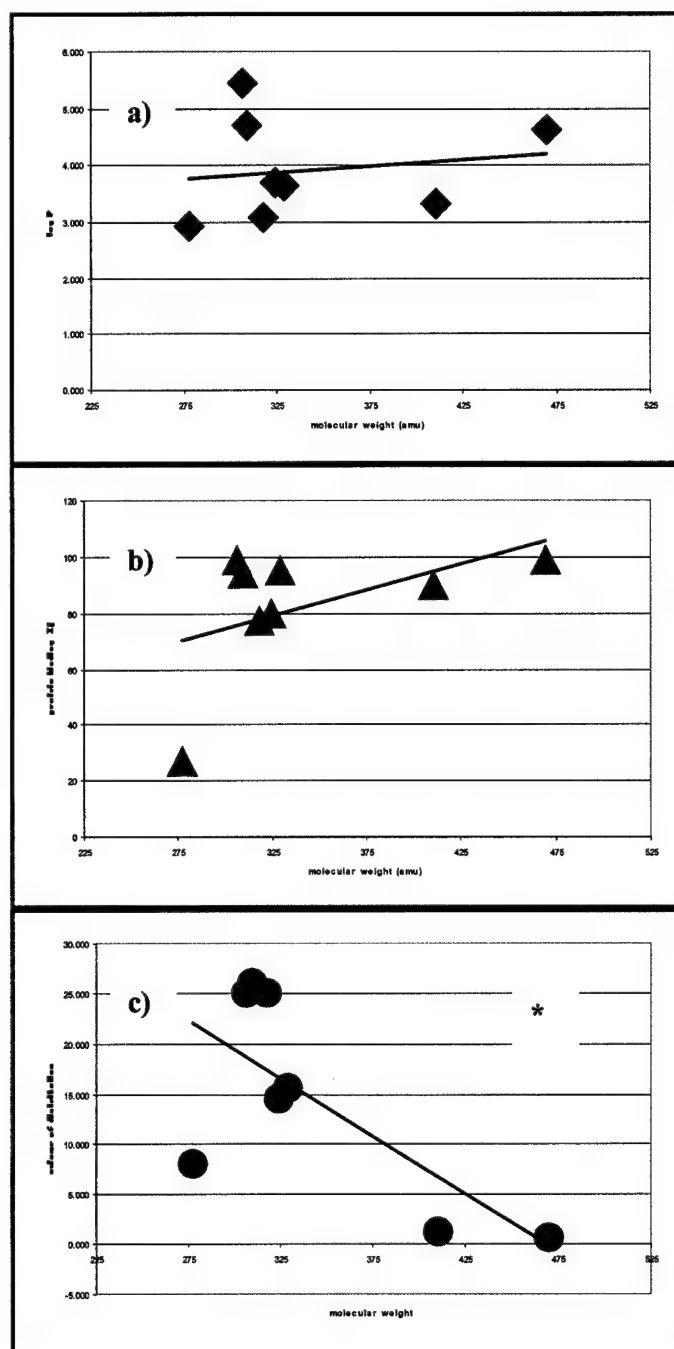
Figure 5.22. Scattergrams showing a) lack of correlation between MW and lipophilicity, b) slight correlation between MW and % protein binding, and c) negative correlation between MW and volume of distribution for the target drugs. $^*p<0.05$.

A positive, significant correlation was observed between vitreous: blood concentrations ratios and those of urine:blood (r2 = 0.760, p<0.01) (see Figure 5.23). This was interesting in light of the high degree of unpredictability in urine concentrations. It may stem from the high water content of both these specimen types. However, there was no significant correlation between urine or vitreous concentration ratios and volume of distribution, lipophilicity or degree of protein binding. Thus it is impossible to make definitive conclusions about either the relationship between these parameters and urine or vitreous concentrations or the meaning of detected urine concentrations.
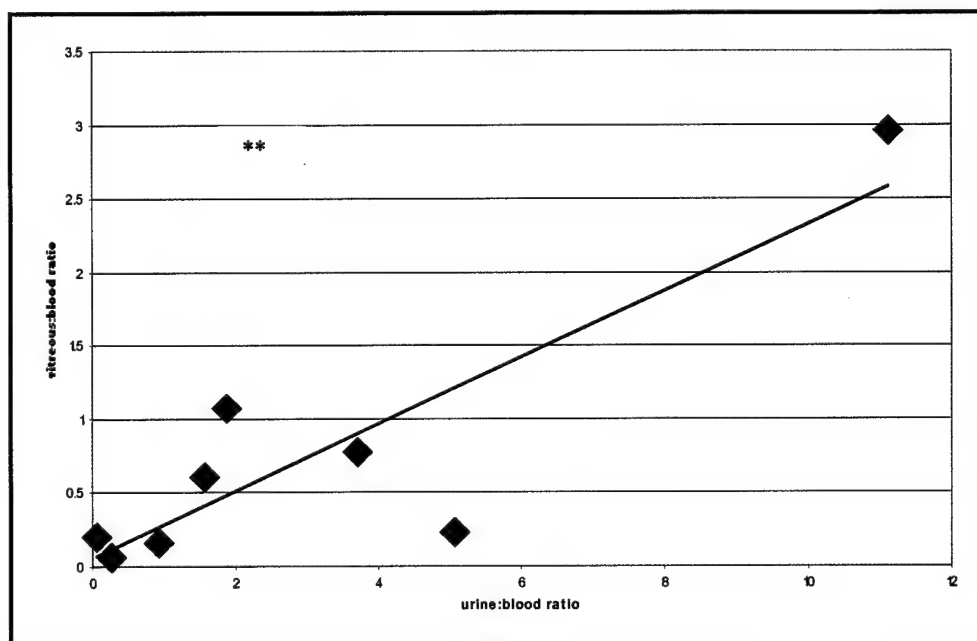
Figure 5.23. Scattergram showing correlation between urine:blood and vitreous:blood
*concentration ratios. ** p<0.01.*

In his study of SSRI tissue distribution, Jaffe theorized that the relatively low vitreous humour concentrations detected for these drugs might be due to the long delay between drug absorption and distribution to this tissue (Jaffe, 1997). Although the author did not measure metabolites in his cases, it was suggested that since SSRI metabolites have longer half-lives than their respective parent drugs, they are likely present in the body for a long enough period to enable distribution into the vitreous humour (Jaffe, 1997). Thus, in cases where low parent drug concentrations were detected in vitreous humour, corresponding metabolite concentrations would be expected to be relatively higher. However, out of the four psychiatric drugs under investigation for which metabolite concentrations were also measured (fluoxetine, risperidone, sertraline, and venlafaxine), metabolite concentrations compared to those of the parent drug in vitreous humour were higher only for O-desmethylvenlafaxine. Whereas vitreous humour concentrations of venlafaxine were the second lowest of all tissues, O-desmethylvenlafaxine concentrations in this tissue were the third lowest. The relative drug concentrations in vitreous humour in comparison to those in blood followed those of their corresponding parent drugs for all other metabolites.

The present results are in accord with those of previously published studies of the tissue distribution of the target psychiatric drugs. In these studies, the highest drug concentrations were detected in liver, brain (for citalopram and fluvoxamine), and bile. The brain regions sampled in these studies were not specified. The lowest concentrations were observed in blood and vitreous humour (Table 1.19) (Anastos et al, in press; Bidanset et al, 1999; Budd and Anderson, 1996; Fu et al, 2000; Jaffe, 1997; Jaffe et al, 1999; Levine et al, 1994; Levine et al, 1996; Logan et al, 1994; McIntyre et al, 1997; Parsons et al, 1996; Robertson, 1999; Singer and Jones, 1997;

Vermeulen, 1998; Worm et al, 1998). However, there were some differences between these results and those in previously published studies. For example, Jaffe found higher venlafaxine concentration ratios for liver compared to bile, whereas in the present results liver ratios were lower. The author also found higher ratios for paroxetine in bile than liver, whereas in the data presented in this chapter, liver concentration ratios were higher than those in bile (Jaffe, 1997). These differences could possibly be due to different types of cases being studied (natural disease, overdoses, etc), although types of death in the published study were not specified.

The authors of the only published study of risperidone tissue distribution detected much higher concentrations in urine than in blood (Springfield and Bodiford, 1996). This was also true for 9-OH-risperidone. In the discussed cases, bile concentrations of both compounds were detected at the highest concentrations. The lowest concentrations for both compounds were detected in vitreous humour and femoral blood. It is impossible to compare these findings to those of Springfield and Bodiford, as they did not analyse bile or vitreous humour specimens. In fact, as of the writing of this dissertation, no information on these tissues with regard to risperidone has been published.

Drug permeation of the blood-brain barrier is difficult to predict. It is dependent on a number of factors, including molecular weight, degree of ionic dissociation at plasma pH, protein binding, and lipid solubility (Cooper et al, 1996). The highest frontal cortex concentrations (normalized for blood concentrations) were for fluoxetine, while the lowest were observed for nefazodone (see Figure 5.20). These findings are probably explained by a combination of the factors mentioned earlier. Finding nefazodone at lower concentrations in the frontal cortex relative to

the other drugs may be due to its higher molecular weight, as compounds with higher molecular weights have more difficulty crossing the blood-brain barrier (Lefauconnier and Hauw, 1984).

Molecular weight only partially explains why frontal cortex concentrations of fluoxetine and sertraline were so high relative to other drugs. Although the molecular weights of both compounds are lower than most other target drugs, they are higher than that of venlafaxine, for which frontal cortex concentrations were considerably lower than in other tissues. The high lipophilicity of sertraline and fluoxetine (logP=5.45 and 4.71, respectively), and the lower lipid solubility of venlafaxine (logP=2.91) may be responsible for these observations (Lefauconnier and Hauw, 1984). However, there was no particular correlation between the different drug concentrations in frontal cortex and either molecular weight or lipophilicity (p>0.05).

Relative degree of protein binding also affects a drug's ability to cross the blood-brain barrier. In a review of antidepressant pharmacology, Frazer noted that the amount of drug present in the extracellular fluid of the brain approximates the amount of non-protein-bound drug concentration in plasma (Frazer, 2001). This may partially explain why risperidone ($F_b = {\sim}0.90$) concentrations in frontal cortex were considerably higher relative to other tissues compared to nefazodone ($F_b = 0.99$), even though risperidone has the second highest molecular weight. Like the other pharmacokinetic parameters already discussed, however, there was no significant correlation between drug concentrations in the frontal cortex and protein binding (p>0.05).

With the exception of fluoxetine, frontal cortex:blood concentration ratios were comparatively higher for the metabolite than for the parent drug. This is an intriguing finding as the metabolites

for the remaining drugs (9-hydroxyrisperidone, N-desmethylsertraline, and O-desmethylvenlafaxine) possess significant pharmacological activity, and generally have longer terminal half-lives than their parent compounds. This suggests the importance of considering active serotonergic drug metabolites when interpreting parent drug concentrations in death investigations. However, the other studies that measured brain concentrations did not measure the metabolites, so it is impossible to compare the present data (Bidanset et al, 1999; Fu et al, 2000).

In general, drug concentrations in vitreous humour and bile were more highly correlated and liver and frontal cortex concentrations least correlated with blood for most drugs (see Table 5.18). The low correlation of frontal cortex concentrations to those of blood may be indicative of either the delay in brain uptake of these drugs due to the presence of the blood-brain barrier or local metabolism. Since psychiatric drug concentrations in liver or frontal cortex are much higher than blood, these tissues could be useful to determine past use. In light of their similar behaviour in relation to blood, it is interesting to note that frontal cortex:blood concentration ratios exhibited a statistically significant, positive correlation with those of liver:blood ($r^2 = 0.681$, $p<0.05$) (Figure 5.24). Frontal cortex:blood concentration ratios were also positively correlated with those of bile:blood ($r^2 = 0.489$, $p>0.05$).

Table 5.18. Summarised correlation data of drug concentrations in postmortem tissues compared to femoral blood[1,2].

Drug	Pattern	Frontal Cortex	Bile	Vitreous	Urine	Liver
Citalopram	B	**0.979**	**0.821**	**0.977**	**0.949**	0.568
Fluoxetine	B	0.177 (0.572)	**0.787** (0.426)	0.051 (**0.666**)	0.000 (**0.905**)	0.263 (0.004)
Nefazodone	D	**0.881**	**0.999**	**0.712**	0.179	0.296
Paroxetine	C	0.018	0.003	**0.749**	0.003	0.113
Risperidone	D	0.070 (0.032)	**0.573** (0.009)	0.347 (0.113)	0.059 (0.049)	0.040 (0.000)
Sertraline	B	0.011 (0.011)	0.004 (0.084)	0.009 (0.066)	0.007 (0.006)	**0.231** (0.002)
Venlafaxine	A	**0.949** (0.018)	0.472 (0.009)	**0.674** (0.223)	**0.664** (0.000)	**0.875** (0.354)

[1] Significant correlations in boldface (p<0.05). [2] Metabolite correlation data expressed in parentheses.

The correlation of tissue metabolite concentrations with those in blood did not follow the same trends as those for their parent drugs. Tissues in which significant positive correlations for parent drugs in blood were observed exhibited much lower correlations for metabolites. Such correlations were seldom significant. This was true for all drugs except venlafaxine. The correlation of liver and vitreous humour concentrations for both venlafaxine and O-desmethylvenlafaxine were significant. The reason for the different behaviour of venlafaxine is unclear, but may be the combined result of this drug's comparatively small extent of protein binding and its lower lipophilicity. As only unbound drug is transported to extravascular sites in the body, venlafaxine concentrations in vitreous humour should agree closely with those in

blood. Although venlafaxine is the most water-soluble of the target drugs, O-desmethylvenlafaxine is even more hydrophilic. It may be that tissue concentrations of these compounds are more reflective of their presence in extracellular fluid than their uptake by tissues, and would suggest that postmortem diffusion from solid tissue into blood is less likely for venlafaxine than for the other drugs under investigation.
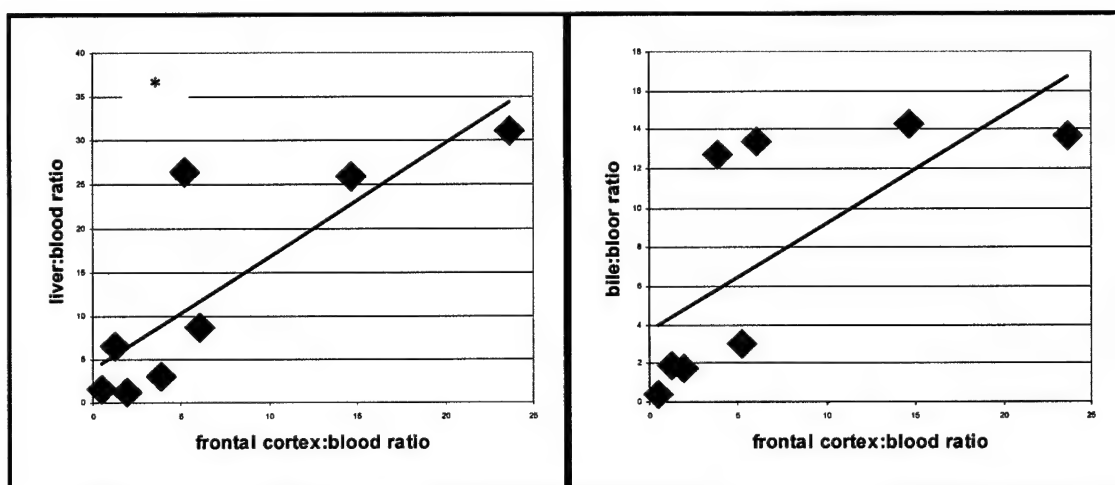
*Figure 5.24. Scattergram showing correlation between frontal cortex:blood and a) liver:blood concentration ratios and b) bile:blood concentration ratios. * p<0.05.*

In summary, drug concentrations detected in tissue specimens from the studied cases followed patterns of distribution which corresponded roughly to drug molecular weight. Molecular weight showed a significant negative correlation with the volume of distribution of the target drugs. Blood concentrations of psychiatric drugs were most highly correlated with those detected in vitreous humour and bile, suggesting these tissues could be useful when blood specimens are unobtainable due to exsanguination or putrefaction. They were least correlated with those in

liver and frontal cortex. However, the concentrations in these tissues were often much higher than those in blood, suggesting their applicability in determining past use of the target drugs.

As stated earlier in this chapter, knowledge of a drug's postmortem tissue distribution pattern is useful in evaluating the degree to which it might undergo redistribution. It is commonly accepted that drugs present in much higher concentrations in tissues than in blood are more likely to undergo postmortem redistribution (Barnhart et al, 2001; Pounder and Jones, 1990; Pounder, 1993; Pounder et al, 1996a; Prouty and Anderson, 1984, 1986, 1989, 1990). The subject of postmortem redistribution of serotonergic drugs will be discussed in Chapter 7.

CHAPTER 6 : BRAIN DISTRIBUTION OF SELECTED ANTIPSYCHOTICS

6.1. Introduction

Antipsychotic drugs are likely to target specific brain regions for their therapeutic effects. However, there is little data in the literature to show the extent to which this occurs in humans (for a review see section 1.6.2 for review).

Brain distribution data for selected antipsychotics reported in the literature is summarised in Table 1.15. While this data is limited, it appears that

a) most antipsychotics partition preferentially into the frontal cortex, midbrain, and caudate-putamen (Aravagiri et al, 1995; Merrick et al, 2001; Svendsen et al, 1988b; van Beijsterveldt et al, 1994), and

b) a degree of differential partitioning between the left and right brain hemispheres occurs (Merrick et al, 2001).

The data from these studies was obtained either from only one subject or from experiments carried out on rats and are therefore of limited value.

How a drug partitions in the brain postmortem would have significant consequences for studies using this tissue to examine target sites for antipsychotic drugs, specifically D_2 and 5-HT_{2A} receptors. Such information could have significant implications for our understanding of how different antipsychotic drugs work and how they produce any toxic outcomes.

From a toxicological standpoint, it is desirable to sample the most appropriate region, if in fact such a region can be established. This region may allow an improved interpretation of possible toxic reactions to such drugs and may also reveal whether antipsychotic drugs follow a general trend in brain distribution or if differences exist between drugs. This would then lead to a common understanding of which brain regions are best to sample for antipsychotic drug determination.

As discussed in section 1.6.1, a number of structural alterations have been observed in the brains of subjects with schizophrenia, including diffuse ventricular enlargement and decreased cortical volume, as well as changes in dopamine concentrations at the cellular level. Since these drugs are thought to impart much of their pharmacological effect through interaction with dopamine receptors (see section 1.3.3), they are commonly administered to schizophrenic patients to restore normal functioning, presumably by normalising dopamine concentrations in the brain.

The purpose of this study was to examine whether selected antipsychotic drugs partition preferentially to particular brain regions in schizophrenic and normal subjects. A further aim was to examine if any correlation exists between brain and blood concentrations.

6.2. Experimental

6.2.1. Materials

Mesoridazine was obtained from the Division of Analytical Laboratories of the New South Wales Health Department (Lidcombe, New South Wales). Sulforidazine and fluphenazine sulfoxide were obtained from the Australian Government Analytical Laboratory (South Melbourne, Victoria). Thioridazine, haloperidol, chlorpromazine, and trazodone, (internal standard), were purchased from Sigma Aldrich Pty Ltd (Castle Hill, New South Wales). Solvents were HPLC grade and were obtained from Sigma Aldrich Pty Ltd. All other chemicals were analytical reagent grade or better and were also obtained from Sigma Aldrich Pty Ltd.

6.2.2. Tissue collection

Caudate-putamen, grey and white matter from the frontal cortex, occipital cortex, and cerebellum were collected at autopsy from 22 subjects with a provisional diagnosis of schizophrenia, and from 11 subjects with no history of psychiatric illness (controls). Dissections were performed by scientists at the Mental Health Research Institute (MHRI), Parkville, Victoria. The tissue was rapidly frozen to -70 °C and stored at this temperature until required.

Sub-cortical structures were identified from an atlas of the human brain. The cortical regions were based on a cytoarchitectural system devised by Brodmann in the early 1900's (Garey, 1994). Caudate-putamen was chosen because of the high concentration of dopamine receptors in

this region and its resulting involvement in schizophrenia (see section 1.6.1 for more information). The frontal cortex is also implicated in schizophrenia, since it is regarded as an area of the brain involved in cognitive function. Differentiation between grey and white matter from the frontal cortex was made to determine whether the antipsychotic drugs accumulate preferentially in grey matter, where the dopamine neurones are located. Similarly, the occipital cortex was included as a form of negative control. It is the cortical region which is not thought to be involved in schizophrenia. Finally, cerebellum was included to investigate whether antipsychotic drug distribution was related to receptor location. Although $GABA_A$ receptors and α_{1A}-adrenoceptors are found in cerebellum, there are very few dopamine or serotonin receptors found there.

Where death did not involve suicide, tissue was collected from subjects whose death was witnessed and the postmortem interval (PMI) was defined as in section 2.4. In suicidal deaths, tissue was only taken from individuals who had been seen alive up to 5 hours before being discovered dead. Again, the PMI was calculated as described in section 2.4. In all cases, the cadavers were refrigerated within 5 hours of being found.

6.2.3. Diagnosis of schizophrenia

The provisional diagnosis of schizophrenia was confirmed by a senior psychologist (Christine Hill) and senior psychiatrist (Professor Nicholas Keks) employed by the MHRI, after an extensive case history review (Hill et al, 1996). These reviews were carried out using a structure instrument called Diagnostic Instrument for Brain Studies (see Keks et al for review) (Keks et al,

1999). In this study all diagnoses were confirmed using DSM-IV criteria (see section 1.6.2) (American Psychiatric Association, 1994). Information on the type and amount of antipsychotic drugs prescribed close to death was obtained from the case history and the final recorded dose of antipsychotic drug was converted to chlorpromazine dose equivalents. Conversion factors determined by consulting published literature sources and psychiatrists for oral and depot neuroleptic preparations have previously been published (see Appendix C) (Foster, 1989). Based on this information, Foster listed the antipsychotic drug dosages shown in Table 6.1 as being clinically equivalent.

Table 6.1. Oral and depot drug equivalents of selected antipsychotics[1].

Drug	Equivalent Dose[2]
Oral preparations	
Chlorpromazine	100 mg
Haloperidol	3 mg
Perphenazine	8 mg
Promazine	100 mg
Thioridazine	100 mg
Trifluoperazine	5 mg
Depot preparations	
Clopenthixol	80 mg
Flupenthixol	16 mg
Fluphenazine	10 mg
Haloperidol Decanoate	30 mg
Pipothiazine	20 mg

[1] (Foster, 1989). [2] Dosages of depot preparations correspond to fortnightly IM injections.

6.2.4. Specimen preparation

Specimens were collected and prepared using the methods outlined in Chapter 2. 1 mL of homogenate was used for the extraction method. Metabolite concentrations of fluphenazine and thioridazine were measured as well as the parent drugs chlorpromazine, trifluoperazine, and thioridazine.

6.2.5. Ethics approval

Ethics approval for the use of human blood was granted by the VIFM ethics committee. The Donor Tissue Bank of Victoria (DTBV) obtained informed consent from the senior next of kin in accordance with procedures outlined in section 2.2.2.

6.2.6. Routine toxicology

Routine specimen preparation and toxicological testing to determine blood concentrations of drugs of abuse and any basic/neutral drugs was carried out using the procedures outlined in Chapter 2. Where screening tests showed the presence of other drugs (including benzodiazepines, opiates, antidepressants, and/ or antipsychotics), confirmation and quantitation was carried out in accordance with section 2.6. Blood alcohol testing was also performed.

6.2.7. LC-MS conditions

Instrumental analysis of prepared specimens was carried out using the instrumental conditions outlined in Chapter 3. For quantitation purposes, mass spectral detection in SIM mode was carried out, monitoring the ions listed in Table 6.2.

Table 6.2. Ions chosen for selected ion monitoring of target neuroleptic drugs.

Compound	Ions Monitored
Chlorpromazine	319, 320, 321
Fluphenazine Sulfoxide	454, 455, 456
Flupenthixol	435, 436, 437
Haloperidol	376, 377, 378
Mesoridazine	387, 388, 389
Sulforidazine	403, 404, 405
Thioridazine	371, 372, 373
Trifluoperazine	408, 409, 410

* The m/z of the base peak for each drug is underlined.

6.2.8. Comparison of analytical results

This method was validated by analysing a series of five replicate brain specimens spiked with 100 ng/mL standards of the target antipsychotics. Results were evaluated on the basis of drug recovery, intra- and inter-assay precision (measured as coefficients of variation, or CV's), and accuracy. Recovery was calculated by comparing the peak area of an extracted brain specimen spiked with a known drug concentration to that of unextracted standards of known concentrations

and expressed as a percentage. CV's were calculated using the standard deviation (σ) and mean (x) of replicate analyses of specimens spiked with known concentrations using the formula CV = (σ/x)·100. Lastly, accuracy was calculated by dividing the measured concentration by the calculated concentration and multiplying the result by 100. The LOQ for mass spectral detection was defined as the concentration that consistently resulted in CV's <20% in SIM mode for all target drugs.

The data from each brain region was normalised for each subject by expressing concentration as a percentage of the cerebellum concentration. Brain distribution of chlorpromazine, fluphenazine sulfoxide, and trifluoperazine was compared for inter-subject variability. Data from each brain region in thioridazine-positive subjects was separated and the parent drug data compared to that of the two principal metabolites, mesoridazine and sulforidazine.

6.2.9. Statistical analyses

Statistical evaluation of this data was performed using SPSS V9.0.1 for Windows software on an IBM personal computer. Parametric Pearson correlation tests at the 95 % confidence interval were used to determine correlation of concentrations measured in each brain region to those in blood for thioridazine and its metabolites and chlorpromazine. Pearson correlation tests were also used to determine the extent of correlation between regional drug concentrations and V_d, extent of protein binding, and lipophilicity.

6.3. Results

6.3.1. Method validation

Within- and between-day coefficients of variation for all drugs analysed were below 10 % at a concentration of 100 ng/g (see Table 6.4). The LOQ for all drugs was 10 ng/g wet weight. Calibration curves passed through the origin and were linear over a concentration range of at least 50-5000 ng/g.

Table 6.4. Mean recovery, accuracy, and precision data for neuroleptic determination in brain[1,2].

Compound	Recovery	Accuracy	Inter-assay CV (N=5)	Intra-assay CV (N=5)
Chlorpromazine	89.2	105	8.76	3.93
Fluphenazine Sulfoxide	77.9	87.5	9.15	1.87
Flupenthixol	82.2	88.1	9.71	9.15
Haloperidol	67.3	104	4.60	1.33
Thioridazine	118	103	6.50	2.74
Mesoridazine	108	82.4	8.67	3.46
Sulforidazine	69.9	82.9	9.53	10.4
Trifluoperazine	79.6	88.6	7.98	3.16

[1] All validation data expressed as percent. [2] Data obtained from blank brain tissue spiked with 100 ng/g drug standards.

6.3.2. Subject demographics

Twenty-two schizophrenic subjects were included in this study. There were approximately twice as many males as females, and the average age of all subjects was 45 ± 4.0 years.

Table 6.5 lists toxicological data in blood of all 22 subjects. Data includes the last recorded antipsychotic drug(s) and, where available, amount of time between the last dose and death.

Unfortunately, the estimated time between dose and death was unavailable in 17 of the 22 cases. In 3 of the 17 cases, no information detailing drug histories was available. In the remaining cases, it was not possible to be certain if the subjects had received medication close to death. However, a low blood concentration of antipsychotic drugs relative to the accepted therapeutic range suggests the possibility that most of the subjects had been noncompliant in the days leading up to their deaths.

Table 6.5. Demographic data, antipsychotic drug history, and tissue collection data from schizophrenic subjects.

ID	Sex	Age (yr)	Cause of Death	Last Recorded Dose of Antipsychotic Drug	CPZ eq[1]	Drugs Detected in Blood	Conc[2]	PMI (h)
1	M	55	coronary arterial thrombosis	thioridazine	400	benzodiazepines (urine)	detected	25
2	F	81	aspiration of food	trifluoperazine	100	thioridazine mesoridazine sulforidazine temazepam	0.2 0.2 0.1 0.1	25
3	M	71	aspiration of food	thioridazine	150	thioridazine mesoridazine sulforidazine diazepam nordiazepam	1.2 0.5 0.2 0.2 0.2	48
4	M	47	ischaemic heart disease	fluphenazine decanoate thioridazine	530	no blood toxicology	--	33
5	M	44	ischaemic heart disease	thioridazine	600	thioridazine mesoridazine sulforidazine	6.8 0.3 0.13	32
6	M	42	drowning	flupenthixol decanoate chlorpromazine	610	no drugs detected	--	35

ID	Sex	Age (yr)	Cause of Death	Last Recorded Dose of Antipsychotic Drug	CPZ eq[1]	Drugs Detected in Blood	Conc[2]	PMI (h)
7	F	48	pulmonary thromboembolism	fluphenazine decanoate chlorpromazine	700	chlorpromazine	0.75	53
						lithium	1.9	
						paracetamol	5.0	
8	F	48	mixed drug toxicity	history refused		chlorpromazine	0.10	65
						ethanol	0.11 g/dL	
						amitriptyline	2.0	
						nortriptyline	1.6	
						clonazepam	0.06	
						7-aminoclonazepam	0.2	
						7-aminoflunitrazepam	0.14	
9	M	29	heroin toxicity	no drug history available		chlorpromazine	0.1	0.0
						morphine	18 urine	
						6-acetylmorphine	0.3 urine	
						codeine	4.0 urine	
						carbamazepine	13	
						paracetamol	3.0	
						cannabinoids	0.05 urine	

ID	Sex	Age (yr)	Cause of Death	Last Recorded Dose of Antipsychotic Drug	CPZ eq[1]	Drugs Detected in Blood	Conc[2]	PMI (h)
10	F	43	Pneumonia	no drug history available		chlorpromazine	2.2	51
						moclobemide	10	
						diazepam	0.1	
						nordiazepam	0.1	
11	F	68	Ischaemic Heart Dis.	trifluoperazine	400	no blood toxicology		42
12	M	65	Bronchopneumonia	trifluoperazine haloperidol decanoate	460	no drugs detected		42
13	M	53	Coronary Arterial Thrombosis	trifluoperazine chlorpromazine	300	diazepam	0.1	
						nordiazepam	0.1	
						paracetamol	5.0	
						fluoxetine	0.1	
						norfluoxetine	0.1	
14	M	41	Mixed Drug Toxicity	fluphenazine decanoate trifluoperazine	500	trifluoperazine	0.11	6.2
						temazepam	4.1	
						diazepam	2.0	
						nordiazepam	0.3	
						dothiepin	6.5	

ID	Sex	Age (yr)	Cause of Death	Last Recorded Dose of Antipsychotic Drug	CPZ eq[1]	Drugs Detected in Blood	Conc[2]	PMI (h)
15	M	22	pericarditis	flupenthixol, 8-days before death; suppl.: trifluoperazine	450	diazepam nordiazepam cannabinoids	trace trace trace	37
16	M	35	perforated gastric ulcer	fluphenazine decanoate	400	no drugs detected		47
17	F	30	hanging	flupenthixol, 4 days before death	600	no blood toxicology	--	48
18	F	35	coronary arterial thrombosis	haloperidol	300	cannabinoids	0.075	15
19	M	38	mediastinitis	haloperidol decanoate	160	morphine diazepam nordiazepam paracetamol frusemide metronidazole	0.2 <0.1 <0.1 3 5 detected	40
20	M	19	unascertained	haloperidol, time of death	750	diazepam nordiazepam	0.1 0.1	43

ID	Sex	Age (yr)	Cause of Death	Last Recorded Dose of Antipsychotic Drug[1]	CPZ eq[1]	Drugs Detected in Blood	Conc[2]	PMI (h)
21	M	23	Multiple Injuries	haloperidol, 1 year, 3 weeks before death	300	diazepam	<0.05	78
						nordiazepam	0.1	
22	M	26	Carbon Monoxide Poisoning	haloperidol decanoate, 2 days before death	500	carboxyhaemoglobin	90% sat	52
						desipramine	0.5	
						7-aminoclonazepam	0.07	
						benztropine	trace	
MEAN		**45**			**430**			**38**
SEM		**4.0**			**45.1**			**3.6**

[1] CPZ eq = chlorpromazine equivalents. [2] Concentrations in mg/L, unless otherwise specified.

Antipsychotic drug concentrations in blood and brains from several of the schizophrenic subjects

(subjects 5, 6, 9, 14, 16, 18-22) were not detected. This includes one trifluoperazine-positive

subject (subject 6), thioridazine-positive subject (subject 9), one chlorpromazine-positive subject

(subject 16), and all of the haloperidol- and flupenthixol-positive subjects (subjects 5, 14, 18-22).

In some of these cases the lack of detected neuroleptics suggest the drugs had not been taken for

some time before death, as the half-lives of the oral forms of fluphenazine, trifluoperazine,

chlorpromazine, and thioridazine are 1-2 days (Baselt and Cravey, 2000). In the case of the high-

potency drug haloperidol and the depot drug flupenthixol, however, the low brain concentrations

detected are more likely the result of therapeutic use rather than non-compliance as blood

concentrations of these drugs are generally much lower than thioridazine and chlorpromazine.

Regardless, such data was not included for comparison between subjects.

6.3.3. Case types and other drugs detected in blood from antipsychotic-positive subjects

There were several cases in which one or more commonly abused drugs were detected. In

particular, benzodiazepines were detected in 11 cases, and opioids and cannabinoids were

detected in two cases each. Antidepressants of varying types (SSRIs, MAOIs, and TCAs) were

present in blood in five subjects.

The manner of death in the studied cases spanned the four major categories. There were 12

deaths ascribed to natural causes, comprising the largest number of cases (subjects 1, 3, 6-8, 11-

15, 18, and 19). There were three accidents (subjects 9, 10, and 21), three suicides not involving

drugs (subjects 2, 17 and 22), and three mixed drug toxicities (subjects 4, 5, and 16). Subject 20's cause of death was unascertainable.

6.3.4. *Regional brain distribution of antipsychotic drugs*

6.3.4.1. Thioridazine distribution

The thioridazine cases revealed the most information out of all antipsychotic drugs studied, since the metabolites mesoridazine and sulforidazine were measured in addition to parent drug in all five thioridazine-positive cases.

Table 6.7 shows a great deal of inter-subject variability in the brain distribution of thioridazine concentrations (highest regional brain concentrations shown in red boldface, the lowest in blue). The relative distribution of thioridazine and metabolites for each subject is shown in Figure 6.1, expressed as % of drug concentration in cerebellum.

Table 6.7. Summary of brain neuroleptic concentrations[1,2].

Subject	CPu	FCx (grey)	FCx (white)	OCx	Cerebellum
			Thioridazine		
1	90 (M: 30, S: 20)	10 (M:10, S: 10)	10 (M:10, S: 10)	20 (M:10, S: 10)	10 (M:10, S: 10)
2	40 (M: 530, S: 250)	240 (M: 10, S: 10)	270 (M: 10, S: 10)	260 (M: 20, S: 110)	210 (M: 10, S: 10)
3	20,000 (M: 2100, S: 610)	3100 (M: 430, S: 20)	1000 (M: 320, S: 10)	2000 (M: 330, S: 10)	1600 (M: 230, S: 10)
4	1200 (M: 430, S: 10)	870 (M: 260, S: 10)	10 (M: 260, S: 10)	10 (M: 260, S: 10)	10 (M: 150, S: 10)
5	9500 (M: 3500, S: 560)	73,000 (M: 21,000, S: 3800)	12,000 (M: 5300, S: 770)	91,000 (M: 26,000, S: 4600)	51,000 (M: 18,000, S: 2900)
			Chlorpromazine		
1	440	580	630	590	310
6	70	70	20	20	10
7	6800	1500	1500	1600	1100
8	1700	10	20	10	10
9	11	<10	<10	<10	<10
10	14,000	14,000	13,000	14,000	9900

Subject	CPu	FCx (grey)	FCx (white)	OCx	Cerebellum
Trifluoperazine					
2	10	100	100	150	100
11	10	1100	580	1200	570
12	10	140	90	190	150
13	10	60	60	60	50
14	9100	9100	5400	9300	8100
Fluphenazine sulfoxide					
15	360	490	400	470	410
16	10	600	470	570	400

[1] Concentrations in ng/mg. [2] CPu = caudate-putamen, FCx = frontal cortex, OCx = occipital cortex.
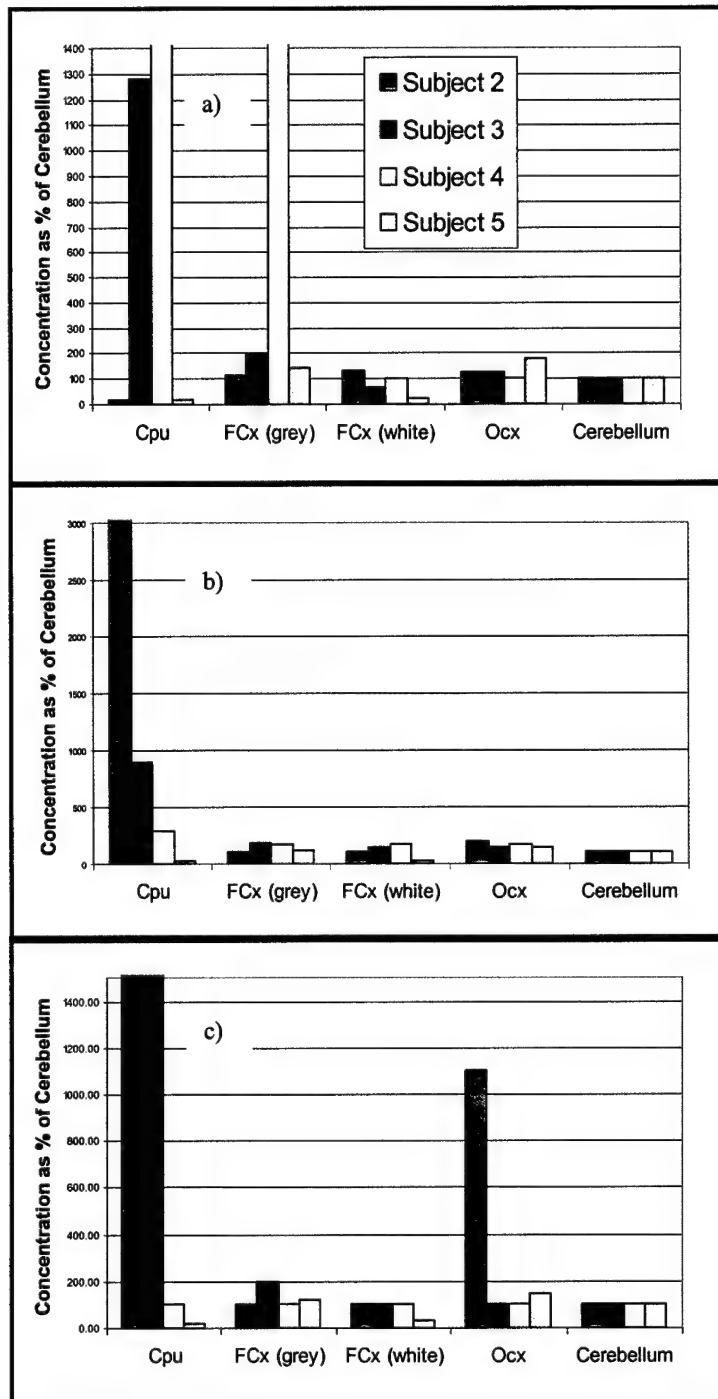
Figure 6.1. Brain distribution of a) thioridazine, b) mesoridazine, and c) sulforidazine in four subjects expressed as % of cerebellum concentration. CPu = caudate putamen, FCx = frontal cortex, OCx = occipital cortex.

In subjects 3 and 4, the highest concentration of thioridazine, mesoridazine, and sulforidazine were detected in the caudate-putamen. This was true for only the metabolites in case 9, the highest parent drug concentration being detected in the white matter of the frontal cortex. This region had the lowest concentrations of each compound in subjects 2 and 5. Because the concentrations of all three compounds were not detected in the remaining regions for subject 1, this case was not included in further analyses. Subject 5 had the highest parent and metabolite concentrations in the occipital cortex, the region with the second highest concentration in the remaining subjects.

6.3.4.2. Chlorpromazine distribution

Brain region concentrations of chlorpromazine detected in subjects 1 and 6-10 are shown in Table 6.7 (highest concentrations in red boldface and lowest in blue). Because chlorpromazine was not detected in any brain regions for subject 9, this case was not included in further data analysis.

Figure 6.2 reveals that like the thioridazine cases, a great deal of inter-subject variability in brain distribution of chlorpromazine was observed. In all subjects positive for the drug, the lowest concentration was found in the cerebellum, and in four of the five remaining subjects the highest concentration was in the caudate-putamen. However, the relative concentrations in occipital cortex and grey and white matter from frontal cortex differed between the subjects.
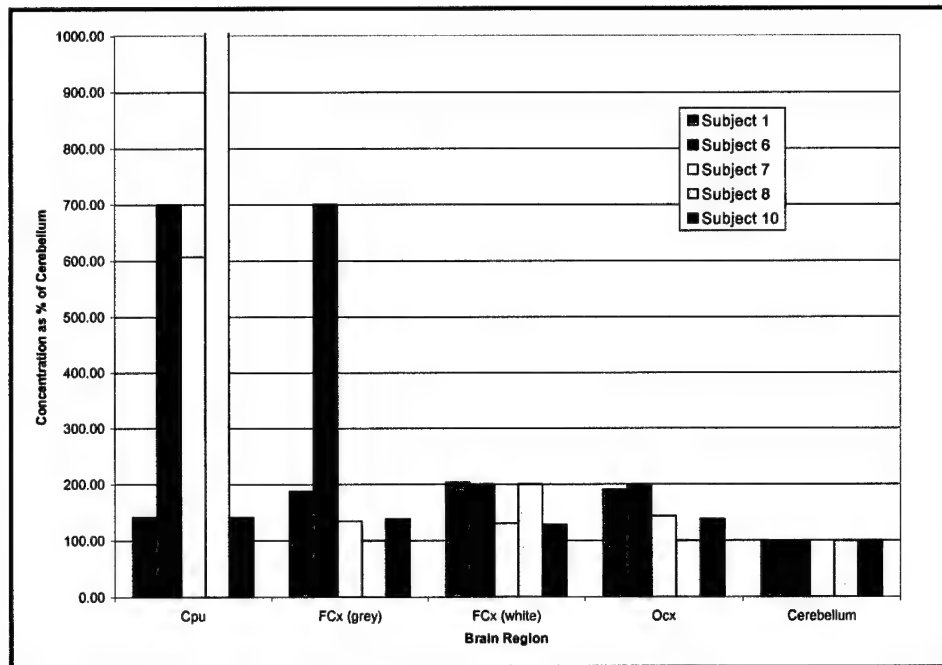
Figure 6.2. Brain distribution of chlorpromazine in four subjects expressed as % of cerebellum concentration. CPu = caudate putamen, FCx = frontal cortex, OCx = occipital cortex.

6.3.4.3. Trifluoperazine distribution

Table 6.7 and Figure 6.3 summarise the regional brain concentrations detected for each trifluoperazine-positive case. Trifluoperazine was found at highest concentration in occipital cortex and was lowest in caudate-putamen, but there was some degree of inter-subject variability in results obtained from grey and white matter from the frontal cortex and the cerebellum.
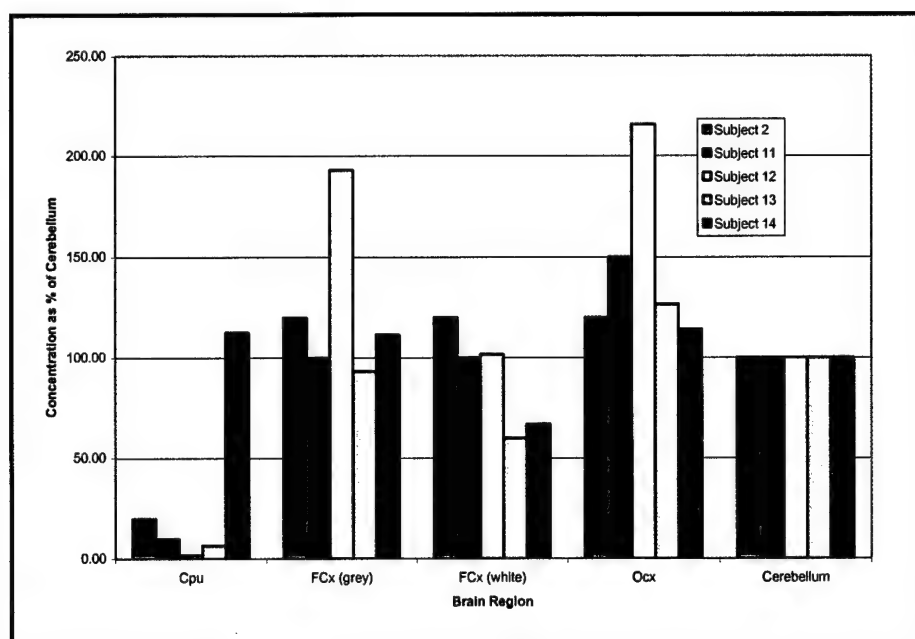
Figure 6.3. Brain distribution of trifluoperazine in five subjects expressed as % of cerebellum concentrations. CPu = caudate-putamen, FCx = frontal cortex, OCx = occipital cortex.

6.3.4.4. Fluphenazine distribution

Since fluphenazine was not available from any Australian supplier, its presence was determined by measuring the concentration of its major metabolite, fluphenazine sulfoxide. This was deemed to be adequate as a study of fluphenazine and metabolite concentrations in rat brain showed fluphenazine sulfoxide concentrations ranged 24-96 % of parent drug concentrations (Aravagiri et al, 1995). As shown in Table 6.7 and Figure 6.4, tissue concentrations in subjects 15 and 16 ranged (from highest to lowest) in the following manner: grey matter from the frontal cortex, occipital cortex, white matter from the frontal cortex/ cerebellum, and caudate-putamen.
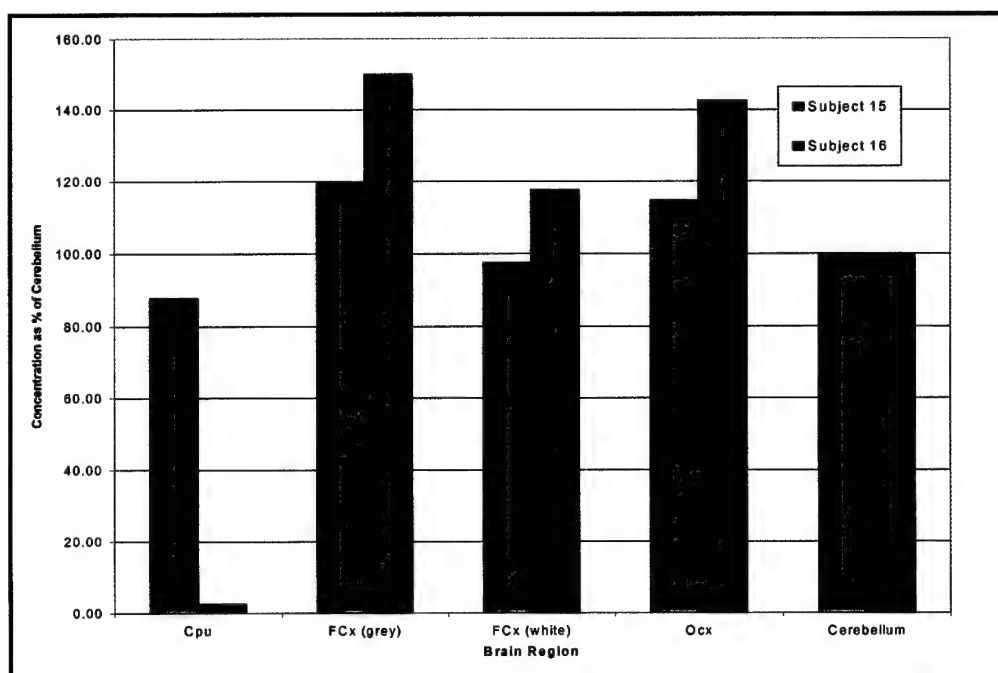
Figure 6.4. Brain distribution of fluphenazine sulfoxide in two subjects expressed as % of cerebellum concentrations. CPu = caudate-putamen, FCx = frontal cortex, OCx = occipital cortex.

6.3.5. Correlation of blood antipsychotic concentrations to those of brain tissue

There were seven cases in which target antipsychotic drugs were detected in both blood and brain (subjects 2, 3, 5, 7, 8, 10, and 14). Of these cases, subjects 7, 8, and 10 were chlorpromazine-positive, subjects 2, 3, and 5 were thioridazine-positive, and trifluoperazine was detected in subject 14. Because of the range of drugs detected, there was insufficient data to test for correlation between blood and brain concentrations for individual drugs. Therefore, the seven cases for which both types of specimens had been analysed were combined and the extent of

correlation between the two specimen types calculated. Significant correlations with blood were observed for concentrations in grey matter of the frontal cortex, occipital cortex, and cerebellum ($p<0.001$) (see Table 6.10 and Figure 6.5).

If combined regional concentrations were expressed in terms of those in cerebellum, much lower correlations were observed, the only significant one being with occipital cortex ($r^2 = 0.783$, $p<0.01$).

Table 6.10. Antipsychotic drug correlation data of pooled brain region concentrations compared to those in femoral blood[1].

Region	Pearson Correlation (r^2)	95% confidence interval for m	p-value
CPu	0.071	-2.408-3.933	0.564
FCx (grey)	0.944	7.646-13.632	0.000
FCx (white)	0.539	-0.109-3.526	0.060
OCx	0.937	9.363-17.287	0.000
Cerebellum	0.927	4.988-9.737	0.001

[1] CPu = caudate-putamen, FCx = frontal cortex, OCx = occipital cortex.

Mean absolute drug concentrations in caudate-putamen exhibited a positive, significant correlation with lipophilicity (measured as logP) ($r^2 = 0.949$, $p<0.05$) (see Figure 6.6). Additionally, when corrected for those in cerebellum, mean drug concentrations in occipital cortex were positively correlated with V_d ($r^2 = 0.994$, $p<0.01$). However, this correlation is likely skewed by the high V_d of fluphenazine (Table 6.11) and therefore not reflective of their actual relationship. No particular correlation between regional drug concentrations and any other parameters were observed.
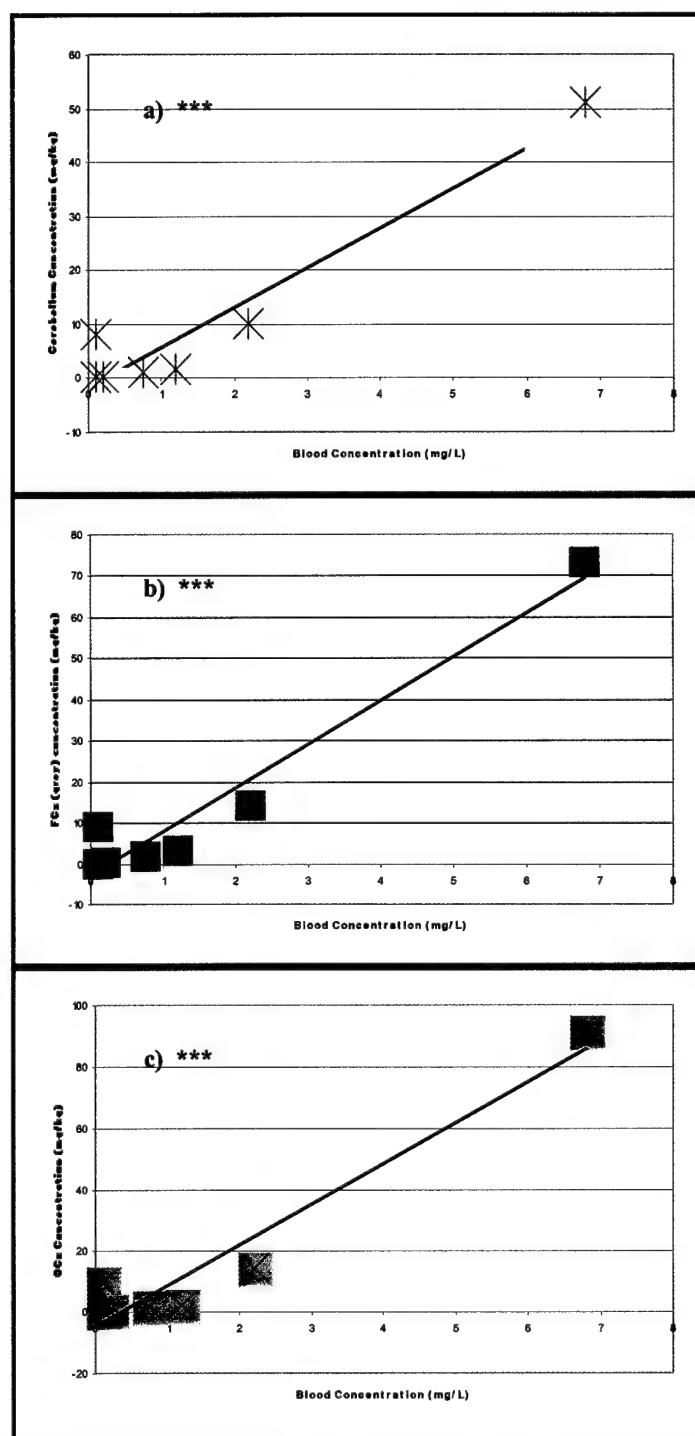
Figure 6.5. Scattergrams showing correlation between blood concentrations and those in a) cerebellum, b) occipital cortex, and c) grey matter from frontal cortex. *** *P<0.001.*

Table 6.11. Mean absolute regional concentrations and pharmacokinetic parameters of psychiatric drugs[1,2].

Drug	CPu	FCx (grey)	FCx (white)	OCx	Cere	pK_a	logP	V_D	F_b
chlorpromazine	3800 ± 5600	2700 ± 5600	5200 ± 2500	2700 ± 690	1900 ± 3900	9.30	4.55	23	0.98
fluphenazine	190 ± 250	550 ± 78	440 ± 49	520 ± 71	410 ± 7.0	8.10	3.46	220	0.99
thioridazine	1800 ± 4100	2100 ± 3900	6200 ± 2300	2200 ± 4000	1800 ± 3500	9.50	5.13	18	0.90
trifluoperazine	6200 ± 8700	15,000 ± 32,000	2700 ± 5200	19,000 ± 40,000	11,000 ± 23,000	8.10	4.26	13	0.95

*

[1] Means ± SD's. Concentrations expressed in ng/g. pK_a's and logP's calculated using CompuDrug Software (CompuDrug Inc., 1999). * $p < 0.05$.
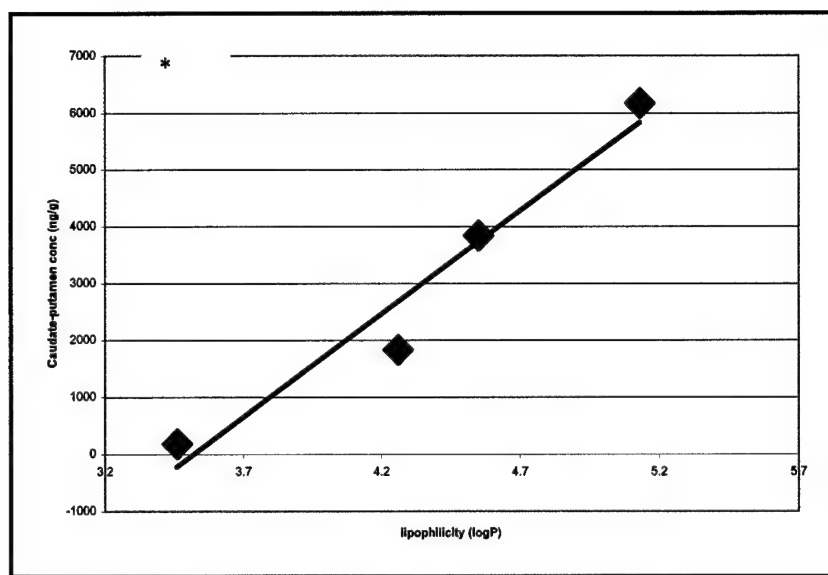
*Figure 6.6. Scattergram showing significant correlation between concentrations in caudate-putamen and drug lipophilicity. * p<0.05.*

6.4. Discussion

6.4.1. Regional brain distribution

The regional brain distribution for each compound using mean absolute concentrations is shown in Figure 6.7. Figure 6.8 shows this distribution with concentrations normalised for those in cerebellum. As shown in these figures, three distinct patterns of distribution were observed in this set of experiments. Chlorpromazine, a low-potency phenothiazine with an aliphatic side chain, was found at highest concentrations in caudate-putamen and lowest in cerebellum (group 1). Thioridazine and its metabolites (group 2) were also detected in caudate-putamen at highest concentrations, while the lowest mean concentrations of these compounds were detected in white matter of the frontal cortex. Phenothiazines in group 2 possess a piperidine ring in the side chain

and are considered atypical antipsychotics because they are associated with fewer extrapyramidal side effects (see sections 1.3.3 and 1.5.2 for discussion of relative phenothiazine potency and side effect profiles). It would appear from my data, therefore, that antipsychotics belonging to groups 1 and 2 accumulate in regions of high dopamine receptor concentration.

The highest and lowest concentrations of both fluphenazine and trifluoperazine (group 3) were found in occipital cortex and caudate-putamen, respectively. Both of these compounds have a piperazine ring in the side chain and are known to possess anticholinergic activity, thus carrying an increased risk of extrapyramidal side effects in comparison to other antipsychotics. The comparatively lower relative concentrations of both fluphenazine sulfoxide and trifluoperazine may be a result of their comparatively lower affinity for dopamine receptors (see Table 1.4) or their higher affinity for peripheral cholinergic receptors. These data partially agree with Aravagiri et al's findings in rat brain, in which the lowest fluphenazine concentrations were found in caudate-putamen (Aravagiri et al, 1995). Like their study in rats, the brain fluphenazine sulfoxide concentrations detected in subjects 15 and 16 showed little regional variation in comparison to the other targeted antipsychotics. However, as was observed in the presented thioridazine-positive cases, it may be that fluphenazine and its metabolites exhibit different regional brain distribution.
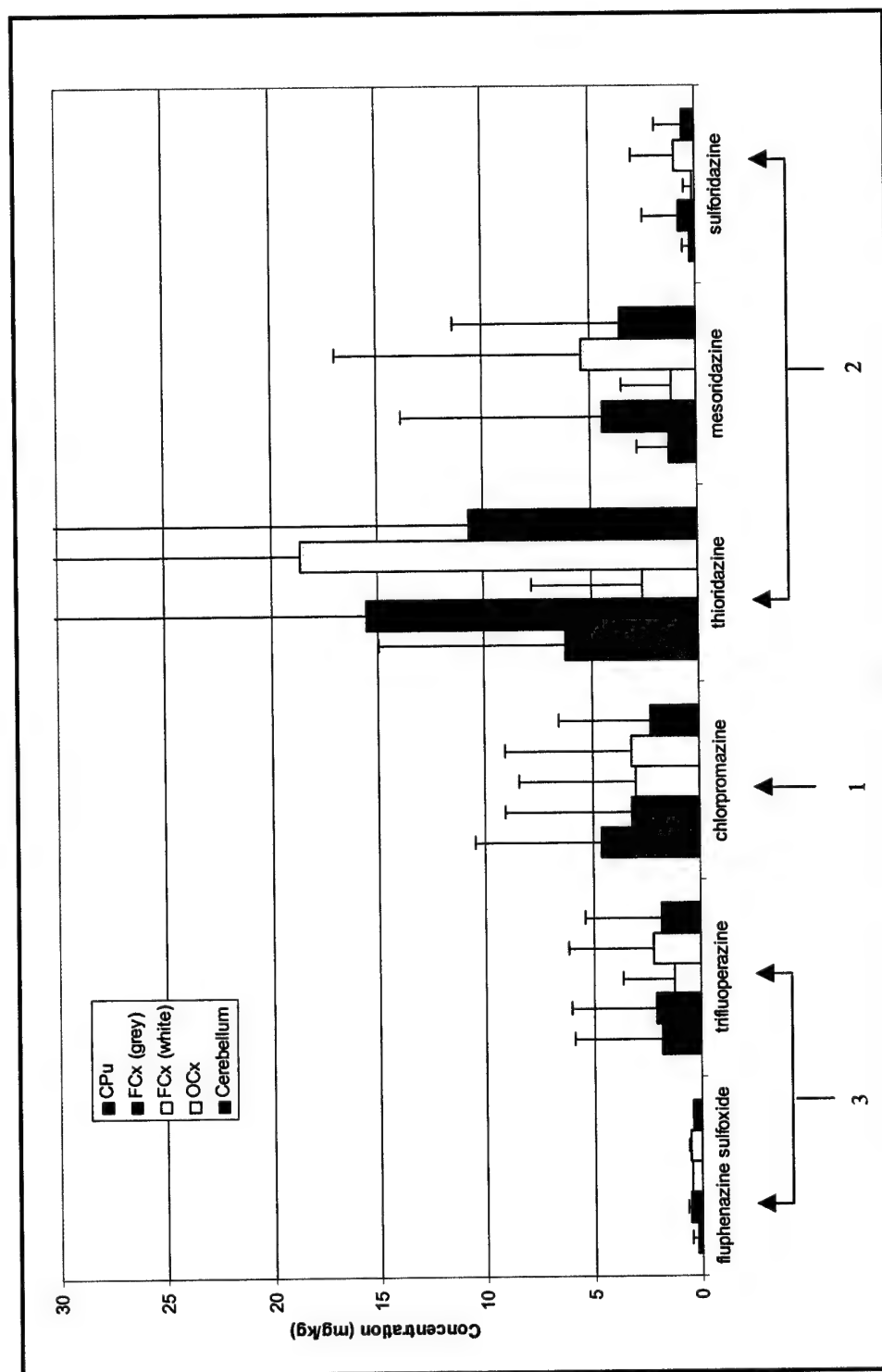
Figure 6.7. Mean absolute regional brain distribution of selected antipsychotic, grouped according to structural features. Group 1: aliphatic side chain, Group 2: piperidine ring in side chain, Group 3: piperazine ring in side chain.
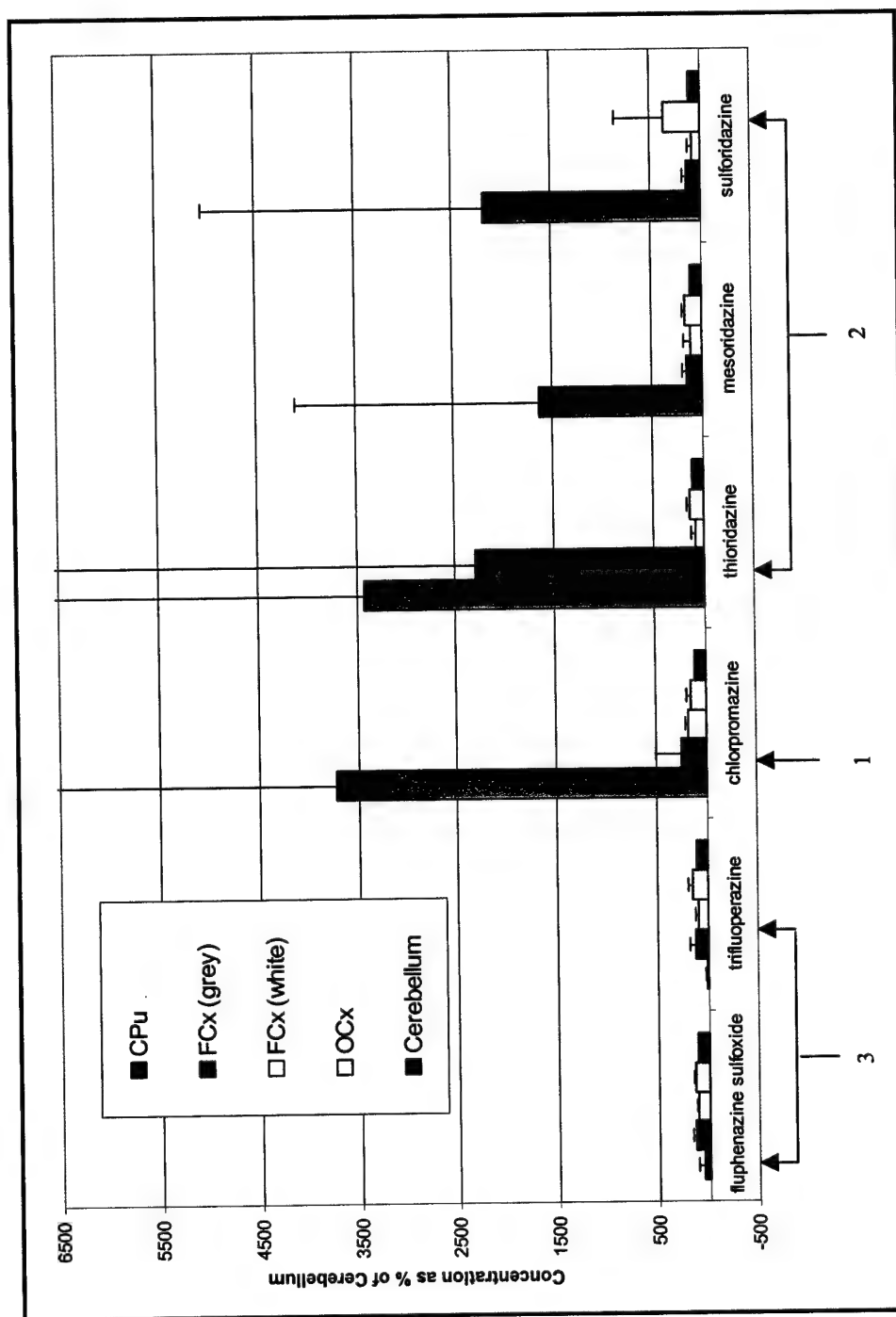
Figure 6.8. Mean regional brain distribution of selected antipsychotics relative to cerebellum concentrations and grouped according to structural features. Group 1: aliphatic side chain, Group 2: piperidine ring in side chain, Group 3: piperazine ring in side chain.

In the presented data, it appears that the regional distribution of thioridazine depends on overall drug concentrations (see Table 6.12). For example, subject 5, who had the highest drug concentrations out of all thioridazine-positive cases, had concentrations in occipital cortex (an area of the brain not thought to be involved in schizophrenia) that were higher than those in other regions. By contrast, subjects 3 and 4, who had moderate overall thioridazine concentrations relative to the other cases, had higher regional concentrations in caudate-putamen (an area of high dopamine receptor concentration). This suggests that antipsychotics first partition into regions where dopamine receptors and their neurones are concentrated, until active sites are saturated. After reaching this point, the drugs may then begin to partition more into regions possessing fewer receptors or neurones or bind to less specific sites.

Table 6.12. Patterns of regional brain distribution for thioridazine.

Pattern	Cases Exhibiting Pattern	Relative sites of concentration (highest to lowest)
A	2	FCx (white)>OCx>FCx (grey)>Cerebellum>CPu
B	3, 4	CPu>FCx (grey)>OCx/FCx (white)/Cerebellum
C	5	OCx>FCx(grey)>Cerebellum>FCx (white)>CPu

It should also be noted that mesoridazine and sulforidazine followed the same pattern of distribution as thioridazine in cases 3-5, but their pattern of distribution differed in subject 9, in which concentrations of all three compounds were significantly lower. This may indicate that although thioridazine and its metabolites appear to distribute in the same way when concentrations are high, the partitioning of each compound begins to differentiate upon cessation of drug use, reflecting a possible differential rate of drug elimination between brain regions.

It is not possible to say whether the variations observed in regional thioridazine distribution in this study are reflective of differences in time between last dose and death, since this information was not available for any thioridazine-positive subjects. Svendsen et al have found that no one area of the brain selectively accumulated thioridazine or its metabolites in subjects known to have taken their medication within 8 hours or less before death (Svendsen et al, 1988b). However, they did find that in one subject who had been taken off medication (800 mg/day) four days before death, there was a more specific distribution of thioridazine and its metabolites in different brain regions. Most significant was the difference between cortical and subcortical structures in which the former had high thioridazine and lower mesoridazine concentrations, whereas the latter had high mesoridazine and lower thioridazine concentrations.

6.4.2. Tissue-to-femoral blood correlations

In all seven cases where both blood and brain tissue specimens were positive for antipsychotic drugs, they were detected at concentrations which were 70 % higher on average in brain than in blood. This is in accord with findings from earlier studies (Aravagiri et al, 1995; Svendsen et al, 1988b). Hence, published data on blood therapeutic and toxic concentrations can generally be expected to be lower than those in brain. However, it was interesting to note that several brain regions (including grey matter from frontal cortex, occipital cortex, and cerebellum) exhibited positive significant correlations with blood ($r^2 > 0.92$, $p < 0.001$). This suggest antipsychotic drug concentrations in these brain regions can be used to predict those in femoral blood, and vice-versa.

It appears from these results that caudate-putamen may be the best region to sample for determination of fluphenazine or trifluoperazine in terms of likelihood of the drug's presence at measurable concentrations. In the case of either chlorpromazine or thioridazine, however, more data is needed to understand what appears to be a complex partitioning profile in postmortem schizophrenic brains.

These data allow two important conclusions to be made. First, low or undetectable concentrations of antipsychotic drug in blood generally correspond to low concentrations in selected regions of the CNS. Second, residual antipsychotic drugs are absent in a large proportion of the brains using detection limits of 10 ng/g (45 %).

It is notable that in many of the cases there was a difference between those drugs detected in blood and their detection in brain. Specifically, although case histories indicated a particular antipsychotic drug should have been present, they were often not detected in blood but often were detected in at least one brain region. This is most likely due to two main factors. Firstly, the brain data was obtained using an LC-MS method that specifically targets the antipsychotic drugs in this study with a low detection limit, whereas the blood toxicology data was collected using older, routine GC screening methods with higher detection limits. Neither the decanoates of flupenthixol and fluphenazine nor haloperidol is detected routinely with these routine GC techniques. Unfortunately, due to time limits imposed on specimen holding, the blood was unable to be re-analysed using the newer LC-MS method. Secondly, in cases where the antipsychotic drug was found in the blood as well as brain, brain concentrations of drug were higher than those found in blood. Therefore, in cases where the antipsychotic was detected in

brain at low concentrations but not detected in blood, its blood concentration may have been below the LOD.

In summary, these data indicate different antipsychotic drugs preferentially distribute to different regions of the brain. Importantly, the distinct patterns of distribution for each of the three types of phenothiazines appear to correspond with their relative affinity for dopamine receptors and the regional brain concentration of such receptors. In addition, it appears from the presented data that regional distribution of antipsychotics may be time-dependent, such that different patterns of distribution are observed at differing points in therapy (commencement of treatment, maintenance, and withdrawal of medication). More data are needed to confirm these findings. Future experiments that focus on the regional distribution of antipsychotics not represented in this study would be especially useful. Additionally, more information about the possibility of differential clearance rates of parent drugs and metabolites from brain tissue would be of value.

CHAPTER 7 : POSTMORTEM REDISTRIBUTION OF PSYCHIATRIC DRUGS

7.1. Introduction

The studies of postmortem redistribution of particular psychiatric drugs reviewed in section 1.7 do not allow a definitive conclusion to be made regarding redistribution of psychiatric drugs in general, or even many of the individual drugs. These studies have shown varying degrees of redistribution for the target drugs as measured by heart:femoral blood drug concentrations. The highest mean ratios have been reported for fluoxetine and fluvoxamine (3.1 for each compound), followed by venlafaxine with a mean ratio of 2.0 for parent drug. The mean ratios for both sertraline and citalopram were the second to lowest (1.3 for each). From these studies paroxetine appeared to undergo the least amount of redistribution, as the mean heart:femoral blood paroxetine concentration ratio (1.2) was the lowest reported for all target drugs (Fu et al, 2000; Jaffe, 1997; Kunsman et al, 1999; Levine et al, 1994; Levine et al, 1996; Logan et al, 1994; Parsons et al, 1996; Roettger, 1990; Rohrig and Prouty, 1989; Vermeulen, 1998). Mean heart:femoral blood metabolite concentrations generally followed those of their parent compounds, with ratios of 1.6, 1.5, and 1.7 for fluoxetine, O-desmethylvenlafaxine (ODV), and N-desmethylsertraline (NDS), respectively.

The uneven tissue distribution patterns of selected drugs observed in the experiments carried out in Chapter 5, combined with the relatively high volumes of distribution (up to 28 L/kg), protein

binding (0.27-0.99), and lipophilicity (logP = 2.91-5.45) of target drugs suggest that postmortem redistribution is very likely. Therefore, in experiments carried out for this chapter, the degree of redistribution was assessed by analysing blood specimens collected from heart and femoral sites. Since the possibility exists for drugs in the stomach contents to diffuse into the liver or centrally-collected blood, this data was compared to drug concentrations measured in liver and stomach contents, when such data were available.

7.2. Experimental

7.2.1. Specimen collection

Matched postmortem femoral and heart postmortem blood specimens were collected in 10 mL plastic tubes containing preservative (1% sodium fluoride and potassium oxalate) and were stored at -20 °C until assay. Specimen collection protocols were carried out as outlined in Section 2.2.

Specimens were classified as to their consistency and colour in the event drug concentrations in blood with a lower than normal haematocrit of 45% (Wintrobe, 1974) might be affected.

7.2.2. Specimen preparation

In all cases where standards were available, relevant metabolite concentrations were measured as well as parent drug. Specimen preparation was carried out in accordance with methods outlined in Section 2.3.

7.2.3. Instrumental conditions

LC-MS analysis of prepared specimens was carried out using the instrumental conditions outlined in Section 3.2.2. Routine toxicological screening was carried out for these specimens in accordance with the procedures outlined in Section 2.5. For quantitation purposes, mass spectral detection in SIM mode was employed, monitoring the ions listed in Table 3.4. After quantitation, heart:femoral blood concentration ratios were determined and checked against recorded observations regarding specimen viscosity and colour.

7.2.4. Postmortem intervals (PMI)

The time of death, time of admission to the VIFM, and time of autopsy were recorded for each case included in this study. The postmortem interval (PMI) was the time between death and autopsy. In cases where the body was found, the time of death was taken as the half-way point between when the subject had last been seen alive and when he or she was found dead.

7.2.5. Statistical analyses

Statistical evaluation of the data was performed using SPSS V9.01 software on an IBM personal computer. Paired-sample T-tests at the 95% confidence interval were used to determine whether differences between heart and femoral blood concentrations were statistically significant. T-tests were also used to determine whether the heart:femoral blood concentration ratios determined in my experiments were significantly different to those determined in previously published studies where blood concentrations from both sites were reported. Pearson correlations were used to determine statistical significance of correlations between heart:femoral blood concentration ratios and each of the following pharmacokinetic parameters: volume of distribution (V_d), protein binding (F_b) and lipophilicity (logP). Values for logP were calculated using PALLAS Expert System V3.0 software (see appendix B for review).

7.2.6. Ethics approval

Ethics approval for the use of human blood was granted by the VIFM ethics committee. The Donor Tissue Bank of Victoria (DTBV) obtained informed consent from the senior next of kin in accordance with procedures outlined in section 2.2.2.

7.3. Results

A total of 13 cases were investigated. The time of death until the time of admission to the VIFM mortuary and the time to autopsy (PMI) are shown in Table 7.1. In 7 cases the exact time of

death was known. The mean time to autopsy in the 13 cases was 64 h (range 42-90 h).

Table 7.1. Elapsed times of death until admission and autopsy in 13 deaths in which psychiatric drugs were detected.

Case Number	Interval		
	Death until admission n=13	Admission until autopsy n=13	Death until autopsy (PMI) n=13
1	6.2	35	42
2	5.8	37	43
3	5.3	41	46
4	5.8	44	50
5	8.8	43	52
6	7.5	46	53
7	12	45	57
8	5.9	70	76
9	6.8	70	77
10	11	68	79
11	4.8	75	80
12	12	71	83
13	5.8	84	90
Mean[1]	7.5± 2.6	56 ± 17	64 ± 17
Range	4.8-12	35-84	42-90

[1] Mean ± standard deviation.

The concentrations of serotonergic drugs in femoral blood are shown in Table 7.2. Data was categorized into 3 groups representing 24 h periods and separated into their drug types. No case had a PMI of 24 hours or less, and the majority of cases had PMIs in the 49-72 h period. Unfortunately, it is difficult to draw any conclusions from this data because there were so few cases falling into each group. However, in the case of both venlafaxine (a serotonin and noradrenaline reuptake inhibitor or SNARI), and risperidone (an atypical antipsychotic), parent and metabolite blood concentrations in cases where the PMI was long tended to be greater than those cases with a short PMI. However, this did not rise to the level of significance for either drug type (p>0.05). There was a great deal of overlap in concentration between the three PMI groups for either parent SSRIs or their corresponding metabolites.

Table 7.2. Psychiatric drug concentrations for different PMI's in 13 femoral blood specimens.

Drug	Case No.	PMI[1] Group A 25-48 h, n=3	Group B 49-72 h, n=4	Group C >72 h, n=5	All Cases Mean ± SD
SSRI parent drugs (n=7)					
Fluoxetine	2	0.14	--	--	0.14
Fluvoxamine	3	0.75	--	--	0.75
Paroxetine	12	--	--	0.73	0.59 ± 0.21
Paroxetine	13	--	--	0.44	0.59 ± 0.21
Sertraline	6	--	0.24	--	0.15 ± 0.13
Sertraline	8	--	--	0.05	0.15 ± 0.13
Citalopram	7	--	1.6	--	1.6
Mean ± SD		0.45 ± 0.43	0.92 ± 0.96	0.41 ± 0.34	0.56 ± 0.53

| Drug | Case No. | PMI[1] | | | All Cases Mean ± SD |
		Group A 25-48 h, n=3	Group B 49-72 h, n=4	Group C >72 h, n=5	
SSRI metabolites (n=3)					
Norfluoxetine	2	0.16	--	--	0.16
NDS	6	--	0.42	--	0.38 ± 0.06
	8	--	--	0.34	
Mean ± SD		0.16	0.42	0.34	0.31 ± 0.13
SNARI parent drug (n=4)					
Venlafaxine	5	--	0.19	--	
	9	--	--	0.80	
	10	--	--	0.05	
	11	--	--	1.1	
Mean ± SD		--	0.19	0.65 ± 0.54	0.54 ± 0.50
SNARI metabolite (n=4)					
ODV	5	--	0.76	--	
	9	--	--	1.1	
	10	--	--	0.30	
	11	--	--	0.92	
Mean ± SD		--	0.76	0.77 ± 0.42	0.77 ± 0.34
Antipsychotic parent drug (n=3)					
Risperidone	1	0.02	--	--	
	4	--	0.36	--	
	8	--	--	0.22	
Mean ± SD		0.02	0.36	0.22	0.20 ± 0.17
Antipsychotic metabolite (n=3)					
9-OH-risperidone	1	0.008	--	--	
	4	--	0.09	--	
	8	--	--	0.25	
Mean ± SD		0.008	0.09	0.25	0.12 ± 0.12
Overall Mean ± SD:					0.42 ± 0.25

[1] PMI: postmortem interval (time between death and autopsy).

7.3.1. Comparison of site of sampling: femoral blood vs heart blood

The concentrations of serotonergic drugs and their metabolites were compared in all 13 matched autopsy femoral and heart blood specimens (Table 7.3). Differences in blood concentrations between sampling sites in cases positive for each particular drug were not statistically significant ($p > 0.05$). When grouped into the following categories: SSRI, SNARI, and atypical antipsychotic, differences in concentration between heart and femoral blood were still not statistically significant for any category. When results for all drugs and metabolites were combined, however, heart blood concentrations were significantly higher (34%) than those measured in femoral blood ($p < 0.05$). Heart:femoral blood concentration ratios ranged from 0.50-6.2, although they averaged between 2-3:1. With the exception of norfluoxetine in case 2, the mean metabolite concentration ratios were similar to those of their parent drugs. In cases 4, 5, and 12, the heart blood specimen was noticeably lighter in colour and therefore thinner than its corresponding femoral blood specimen. Cases 4, 5, and 12 accounted for the highest heart:femoral blood concentration ratios.

Table 7.3. Drug concentrations in postmortem heart and femoral blood specimens in 13 cases[1].

Drug	Case No	Heart	Femoral	Ratio
SSRI parent drug				
Fluoxetine	2	0.93	0.15	6.2
Fluvoxamine	3	0.59	0.75	0.79
Sertraline	6	0.36	0.24	4.8
	8	0.05	0.05	1.0
Citalopram	7	1.8	1.6	1.1
	12	3.2	0.73	4.4
Paroxetine	13	0.49	0.44	1.1
Mean ± SD		1.1 ± 1.1	0.57 ± 0.53	
SSRI metabolites				
Norfluoxetine	2	1.33	0.16	33
NDS	6	0.93	0.42	2.2
	8	0.26	0.34	0.76
Mean ± SD		0.84 ± 0.54	0.31 ± 0.13	
SNARI parent drug				
Venlafaxine	5	0.28	0.19	5.5
	9	0.72	0.80	0.90
	10	0.05	0.05	1.0
	11	1.1	1.1	1.0
Mean ± SD		0.54 ± 0.47	0.54 ± 0.50	
SNARI metabolite				
ODV	5	0.61	0.76	0.80
	9	1.0	1.1	0.91
	10	0.78	0.30	2.6
	11	1.4	0.92	1.5
Mean ± SD		0.95 ± 0.34	0.77 ± 0.34	

Drug	Case No	Heart	Femoral	Ratio
Antipsychotic parent drug				
	1	0.01	0.02	**0.50**
Risperidone	**4**	**1.8**	**0.36**	**5.0**
	8	0.33	0.22	**1.5**
Mean ± SD		**0.71 ± 0.95**	**0.20 ± 0.17**	
Antipsychotic metabolite				
	1	0.01	0.008	**1.3**
9-OH-	**4**	**0.18**	**0.09**	**2.0**
risperidone	8	**0.50**	**0.25**	**2.0**
Mean ± SD		**0.23 ± 0.25**	**0.12 ± 0.12**	

7.3.2. Redistribution from the gastrointestinal tract

Stomach contents were collected and assayed for psychiatric drug residues in 7 cases, with

absolute amounts ranging from 0.05-2.0 mg. These values are all below the amount of drug

contained in a tablet; hence it was unlikely that significant diffusion would occur from the

gastrointestinal tract to centrally collected blood (Table 7.4). With the exception of cases 2 and

4, the heart blood concentrations were not much higher than those in femoral.

Table 7.4. Concentrations of selected psychiatric drugs in heart and femoral blood and amounts of corresponding drug residues found in stomach contents.

Case	Drug	Heart:Femoral Ratio	Stomach Contents[1]
SSRIs			
2	Fluoxetine	6.2	1.9
3	Fluvoxamine	0.79	0.6
8	Sertraline	1.0	2.0
SNARIs			
5	Venlafaxine	1.5	1.5
10	Venlafaxine	1.0	0.3
Atypical Antipsychotic			
1	Risperidone	0.5	0.1
4	Risperidone	5.0	1.0
8	Risperidone	1.5	1.5

[1] Drug residues in whole stomach contents expressed as mg.

7.3.3. Redistribution from the liver

Liver specimens were collected and analysed for psychiatric drug concentrations in 10 cases, with drug concentrations ranging from 0.05-13 mg/kg. These cases were divided into two groups: those with liver drug concentrations >1.0 mg/kg (category 1), and those with liver drug concentrations <1.0 mg/kg (category 2). The heart:femoral blood concentration ratios in category 1 were compared to the corresponding ratios in category 2 for each parent drug or metabolite detected in each specimen. This accounted for a total of 20 data points. A slight difference in heart blood concentrations between the two categories was observed, although this difference was not statistically significant (Table 7.5).

Table 7.5. Heart:femoral blood concentration ratios for selected psychiatric drugs and metabolites in cases either found, or not found, to contain the same drugs in liver[1].

Case No	Liver Conc	Ratio	Case No	Liver Conc	Ratio
Cat 1: Liver Drug Conc. > 1.0 (n=11)			*Cat 2: Liver Drug Conc. < 1.0 (n=9)*		
2	Fluoxetine 3.9	6.3	1	Risperidone 0.05	0.56
	Norfluoxetine 2.4	8.4		9-OH-risperidone 0.05	1.3
3	Fluvoxamine 12	0.79	4	Risperidone 0.98	5.0
				9-OH-risperidone 0.10	2.0
5	ODV 1.3	0.80	5	Venlafaxine 0.08	1.5
8	Sertraline 3.3 NDS	1.0	8	Risperidone 0.22	1.5
	13	2.2		9-OH-risperidone <0.02	2.0
9	Venlafaxine 2.5	0.90	10	Venlafaxine 0.08	1.0
	ODV 3.1	0.94			
10	ODV 2.2	2.6	11	Venlafaxine 0.85	1.0
11	ODV 1.6	1.6			
12	Paroxetine 3.1	4.4			
Mean ± SD		**1.2 ± 0.63**	**Mean ± SD**		**2.2 ± 1.8**

[1] NDS = N-desmethylsertraline; ODV = O-desmethylvenlafaxine.

7.4. Discussion

From these results it appears the target psychiatric drugs undergo some postmortem redistribution based on the general trend for higher concentrations in heart blood compared to those in femoral blood. Therefore, it is important to understand through which mechanism(s) this occurs. Following is a discussion of factors that may contribute to this effect.

7.4.1. Differences in extent of redistribution between individual drugs

Insufficient data was obtained to comprehensively determine if particular psychiatric drugs exhibit more or less postmortem redistribution than others, although some information can be gleaned from observed trends. The highest heart:femoral ratios were observed with fluoxetine, paroxetine, and risperidone. The heart:femoral concentration ratios of norfluoxetine and 9-OH-risperidone followed those of their parent drugs. The volumes of distribution of both fluoxetine and paroxetine are quite high and thus these drugs might be expected to undergo postmortem redistribution. This, combined with fluoxetine's high degree of lipophilicity, could explain the high ratios observed in Case 2. More cases are necessary to confirm this finding, although it does accord with observations in two other reports (Roettger, 1990; Rohrig and Prouty, 1989) (see Figure 7.1).   In a study of the tissue distribution and postmortem redistribution of selected SSRIs, Jaffe found small increases in fluoxetine concentrations in the postmortem interval, combined with a slight decrease in norfluoxetine concentrations in specimens stored at 4 °C (Jaffe, 1997). He concluded that no redistribution had occurred in his fluoxetine-present cases, although he conceded his observations might not be reflective of the full picture as postmortem redistribution has been observed in dogs (Pohland and Bernhard, 1997). This was evidenced by decreases in lung and liver concentrations accompanied by increases in heart blood concentrations over time.

7.4.2. Differences in specimen haematocrit

It was notable that in cases 4, 5, and 12 the heart blood specimens were visibly thinner (observed by relative lightness of colour and viscosity) than the corresponding femoral blood specimens. The psychiatric drugs considered in this dissertation are all fairly lipophilic, with logP values ranging 2.91-5.45 (Table 7.6). Lipophilicity enables drugs to permeate cell membranes, so the psychiatric drugs under investigation likely distribute to a large extent into red blood cells. This would not explain the significantly higher heart blood concentrations in the blood samples observed to have lower haematocrit. Delta-9-THC, a highly lipophilic drug (logP=5.78) (Mason and McBay, 1985), has been detected at an average haemolysed blood:plasma concentration ratio of 0.46, matching the expected ratio of red blood cell to whole blood volumes (Mason et al, 1983). In effect, in these studies plasma Δ^9-THC concentrations were diluted by the presence of red blood cells. In the case of the target psychiatric drugs, however, evidence suggests that plasma SSRI concentrations more accurately reflect those of whole blood than either Δ^9-THC or the tricyclic antidepressants (Amitai et al, 1993). Therefore, the reason for the anomalous drug concentrations in cases 4, 5, and 12 is unclear.

In one of two published studies of paroxetine tissue distribution in humans, paired central and peripheral blood specimens were collected in one case. However, the peripheral blood specimen was aortic so the central:peripheral blood ratio was not reflective of the drug's concentration in femoral blood (Vermeulen, 1998) (see Figure 7.1). In his study of selected SSRIs, Jaffe had conflicting data from paroxetine-positive cases, and thus was unable to draw firm conclusions from these cases. The interpretation of data is further complicated by a study of postmortem

redistribution of paroxetine in the rat which showed increases in drug concentrations in

postmortem blood accompanied by decreased drug concentrations in the lung over the

postmortem interval (Kupiec et al, 1998).

Table 7.6. Volumes of distribution (V_d), % protein bound (F_b) and lipophilicity (logP) values for drugs under investigation[1].

Compound	V_d(L/kg)	F_b	logP
Citalopram	15	0.80	3.68
Fluoxetine	26	0.94	4.71
Fluvoxamine	25	0.77	3.08
Nefazodone	0.55	0.99	4.63
Paroxetine	16	0.95	3.63
Risperidone	1.2	~0.90	3.33
Sertraline	25	0.99	5.45
Venlafaxine	8	0.27	2.91

1 logP values calculated using Pallas 3.0 Software from CompuDrug Int'l (CompuDrug Inc., 1999).

No significant difference in concentration was observed for citalopram, sertraline, or venlafaxine in blood collected from heart versus femoral regions. The mean sertraline heart:femoral concentration ratio measured in my experiments was significantly higher (39%) than those reported in other studies ($p < 0.05$), although other studies also reported no significant difference in heart versus femoral blood sertraline concentrations (Levine et al, 1994; Logan et al, 1994) (see Figure 7.1).
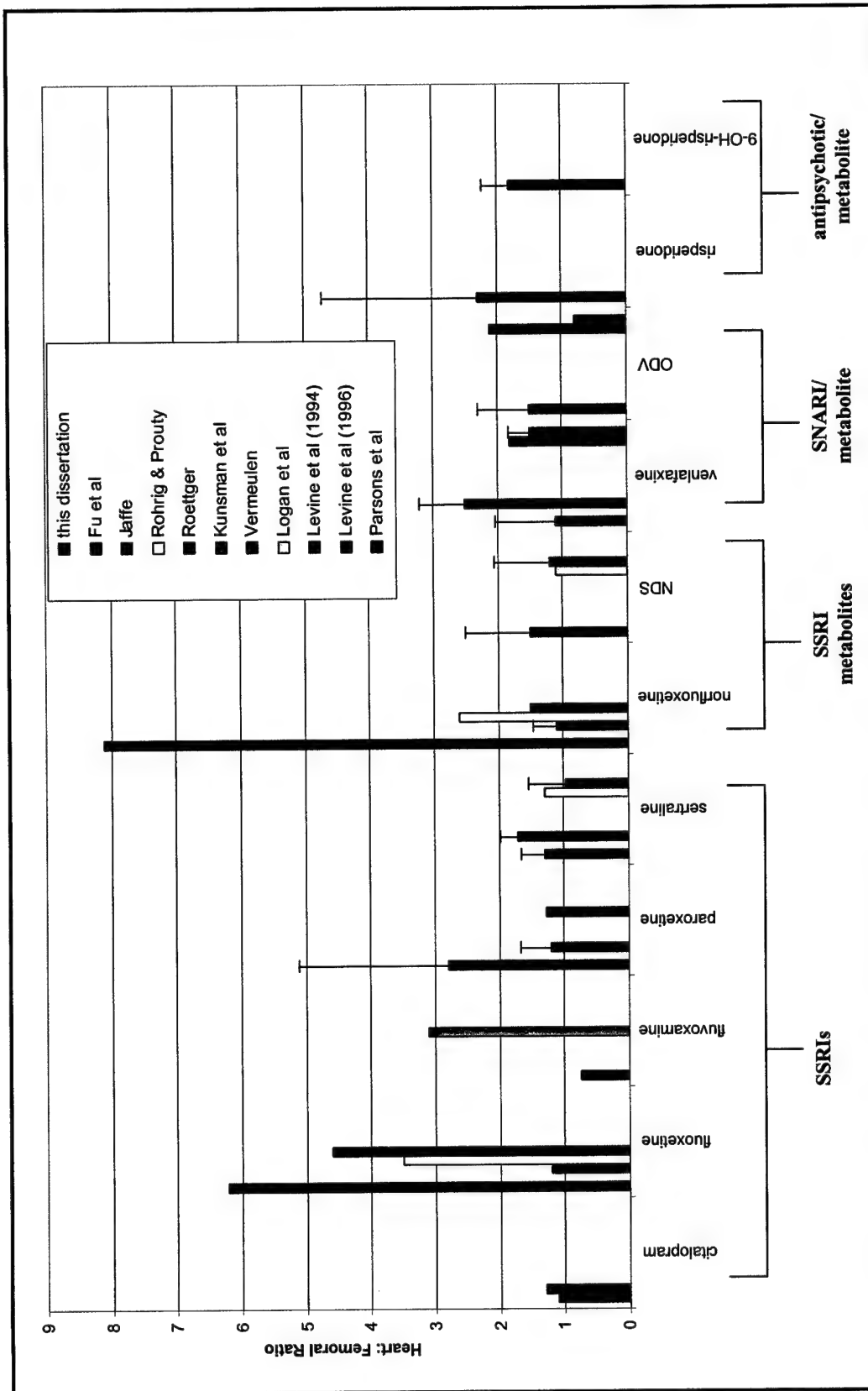
Figure 7.1. Heart: femoral concentration ratios in this dissertation and in selected references. Data for metabolites included from studies in which their concentrations were also reported.

Other studies on citalopram and venlafaxine have found higher heart:femoral concentration

ratios, more in line with that measured in case 5 (Fu et al, 2000; Levine et al, 1996; Parsons et al,

1996) (see Figure 7.1). However, the citalopram, venlafaxine, and O-desmethylvenlafaxine

concentrations measured in other studies were not significantly different to those measured in my

experiments for either parent drug or metabolite (p>0.05). My findings are consistent with both

the lower volumes of distribution and protein binding for citalopram and venlafaxine compared

to the other drugs. The heart:femoral concentration ratio of O-desmethylvenlafaxine was higher

than that of venlafaxine (1.5:1 compared to 1.1:1), so the difference in ratios between my results

and those of other studies may be attributable to differences in parent drug:metabolite ratios.

Jaffe found venlafaxine to be the only drug he studied which exhibited significant postmortem

redistribution, evidenced by an average autopsy:admission blood concentration ratio of 2.5:1

(Jaffe, 1997) (see Figure 7.1). This finding was surprising in light of venlafaxine's

comparatively low volume of distribution. Venlafaxine is the least lipophilic psychiatric drug

studied, and exhibits the smallest degree of protein binding out of all psychiatric drugs under

investigation. These differences between studies might also reflect the variability that exists

between cases.

In my cases, fluvoxamine was present at higher concentrations in femoral blood compared to

heart blood. These findings do not accord with those of the only other published report

discussing postmortem redistribution of fluvoxamine, in which the heart:femoral blood

concentration ratio was about 3.1:1 (Kunsman et al, 1999) (see Figure 7.1). Studies have shown

arterial-venous differences in drug concentrations during the drug absorption and distribution

phase (Pounder, 1993). Therefore, if a person dies while a drug is still being distributed

throughout the body, toxicological results may vary and may be impossible to predict. This may explain the lower heart:femoral ratios observed in my data for Case 3. Since fluvoxamine acid concentrations were not measured in this case, it is not possible to make conclusions regarding the extent of antemortem drug absorption or distribution.

7.4.3. *Factors contributing to postmortem redistribution*

It has been suggested that in some cases of drug overdose, a certain amount of unabsorbed drug remains in the stomach and gastrointestinal tract (GIT), which can diffuse into abdominal blood vessels postmortem. This would result in an increase in drug concentrations during the postmortem interval (Hilberg et al, 1992b). For this reason, I investigated whether the presence of psychiatric drugs in the gastrointestinal tract could be linked to the finding of statistically significant differences in heart vs. femoral blood concentrations. Unfortunately, however, the drug residues measured in the stomach contents in my cases were small, that is, less than the amount of in a tablet for each given drug. Because of this, it was difficult to determine if diffusion of psychiatric drugs from the GIT postmortem contaminates centrally-collected blood.

A large portion of the liver, particularly the right lobe, is directly adjacent to the stomach. It is possible therefore that in cases where large amounts of drug are detected in the stomach, some drug may diffuse into the right lobe of the liver, thereby increasing the measured concentration in this tissue. In postmortem studies of amitriptyline redistribution in the rat, Hilberg et al observed drug diffusion from the stomach to the liver lobes adjoining the stomach in specimens taken five hours after death (Hilberg et al, 1992a). Amitriptyline concentrations throughout the liver

increased with time, although this increase was smaller in the lobes which are further away from the stomach. Pounder has published similar data from a human cadaver model, in liver specimens taken from regions nearer the stomach showed higher concentrations of paracetamol, amitriptyline, and lithium after directly administering them into the stomach (Pounder et al, 1996b). In another case, in which the person died following a zopiclone overdose, eight samples from different areas of the liver were obtained at autopsy 96 hours after the body was discovered. The highest drug concentrations were found in those areas directly in contact with the stomach. The bile, in which zopiclone was measured at a concentration of 14.1 mg/L, also seemed to have an effect on liver concentrations, as the highest drug concentrations were measured in regions of the liver adjacent to both the stomach and the gall bladder. Because drug residues in the stomach contents in my cases were small, it is impossible to say whether psychiatric drugs diffused from the GIT to the liver.

The extent to which a drug undergoes postmortem redistribution is dependent on a number of physicochemical properties, including volume of distribution, protein binding and lipophilicity. Table 7.6 lists these parameters for each psychiatric drug under investigation. In general, drugs with high volumes of distribution are more likely to undergo redistribution (Barnhart et al, 2001; Pounder and Jones, 1990; Pounder, 1993; Pounder et al, 1996a; Prouty and Anderson, 1984, 1986, 1989, 1990). Degree of protein binding and lipophilicity also contribute to redistribution in that they partially determine the extent to which drugs concentrate in certain tissues. High lipophilicity permits drugs to rapidly permeate cell membranes so they may distribute and be taken up by tissues and concentrate in body fat (Pounder, 1993).

Pearson nonparametric tests were conducted to determine the statistical significance of correlations between heart:femoral blood concentration ratios and each of the following pharmacokinetic parameters for the drugs under investigation: volume of distribution, protein binding, and lipophilicity. No significant correlation was observed between concentration ratios and any of these parameters, whether results for drugs were categorised according to drug type (SSRI, SNARI, antipsychotic) or results for all drugs were tested together in one group ($p > 0.05$). If results from previously published studies were also included (Fu et al, 2000; Jaffe, 1997; Kunsman et al, 1999; Levine et al, 1994; Levine et al, 1996; Logan et al, 1994; Parsons et al, 1996; Roettger, 1990; Rohrig and Prouty, 1989; Vermeulen, 1998), correlations still did not rise to the level of significance. Because of this, it appears that although each of these parameters may partially affect drug concentrations in postmortem blood, some other factor may come into play that affects postmortem concentrations of psychiatric drugs. This might explain why, for example, no significant differences between heart and femoral blood concentrations were observed in my sertraline-positive cases even though it has a large volume of distribution (25 L/kg), degree of protein binding (99%), and lipophilicity (logP=5.45). Variability between cases could also be a factor, or blood being taken from a femoral site without first ligating the vessel, resulting in blood specimens that were not truly femoral in origin.

The diffusion of drug along a concentration gradient, i.e. from solid organs in which drugs are known to concentrate to blood, has been implicated in the phenomenon of postmortem redistribution. Liver was included in these experiments because it is a solid organ and therefore high drug concentrations in this tissue may reflect high concentrations in other solid tissues.

The fact that a significant difference was seen in heart:femoral blood concentration ratios between Categories 1 and 2 (Table 7.5) may mean postmortem drug diffusion from solid tissue to blood does occur, leading to some redistribution.

Having excluded significant contribution from either reabsorption from the GIT or differences in haematocrit, my observations indicate redistribution of psychiatric drugs appears to occur, a process likely driven for the most part by diffusion of drug from tissues and solid organs containing high drug concentrations to areas of lower concentration. Femoral blood concentrations were generally higher for specimens with longer PMI's, although this increase was not statistically significant. There was, however, a general trend for higher concentrations in heart blood compared to femoral blood which was statistically significant when results for all serotonergic drugs tested were included together ($p < 0.05$). This observation highlights the fact that even femoral blood concentrations can be elevated over the course of the PMI. Because of this, antemortem or admission blood specimens are important in assessing overall extent of redistribution.

More data is needed to evaluate the degree of variation in redistribution attributable to each drug. Additionally, further experiments comparing drug concentrations in blood taken at time of admission to those obtained in blood taken at autopsy might help to evaluate the extent of diffusion that occurs. Regardless, the data obtained in this set of experiments demonstrates that postmortem redistribution is important to consider when interpreting psychiatric drug concentrations measured in blood, and therefore site of collection of blood specimens should be standardized and noted at autopsy.

CHAPTER 8 : TRENDS IN CAUSE OF DEATH

8.1. Introduction

The psychiatric drugs studied in this dissertation are all capable of causing adverse medical events if misused or taken in combination with other drugs. However, it is not always clear what role a drug plays in a particular death when the case circumstances do not clearly show acute intoxication (such as a suicide). The interpretation of potential drug deaths has become more difficult with the arrival on the market of relatively new serotonin-active drugs that have a potential for causing adverse interactions with other drugs.

An investigation was therefore undertaken to investigate the toxicology of the target psychiatric drugs in a series of fatalities. The ultimate objective of this investigation was to highlight possible risk factors associated with the use of such drugs.

8.2. Experimental

8.2.1. Case examination

Case information from police, toxicology, and pathology reports, as well as coronial findings and inquests was obtained from deaths occurring between October 1999-April 2002 in which

specimens had been collected to study tissue distribution (discussed in Chapter 5). This

information was examined and the circumstances of the cases collated.

Deaths were grouped into the following types: natural, non-drug accidents (such as a fall or a

complication of surgery), non-drug suicides (such as drowning, carbon monoxide poisoning, or a

hanging), or drug-related deaths (whether accidents or suicides). Deaths in each group were

investigated for toxicological significance. Each case was examined to investigate the possible

occurrence of serotonin syndrome, neuroleptic malignant syndrome, or other serotonin-related

medical adverse events and for possible interactions leading to cytochrome P450 (CYP450)

enzyme inhibition.

8.2.2. Determination of relative drug contribution to death

In order to estimate the relative contribution of each target drug to death, the detected

concentration was compared to ranges of concentrations in postmortem blood previously deemed

to have arisen from therapeutic use. This was based on data derived from approved compilations

such as USPDI and MIMS as well as case reports from the literature (Anastos et al, in press;

Baselt et al, 1978; Baselt and Cravey, 2000; Budd and Anderson, 1996; Caswell and et al, 2001;

Goeringer et al, 2000a; Grant and Finton, 1994; Greene and Barbhaiya, 1997; Gupta and

Dziurdy, 1994; Jaffe et al, 1999; Jann et al, 1985; Jann, 1991; Joffe et al, 1998; Jorgensen et al,

1982; Kunsman et al, 1999; Levine et al, 1994; Logan et al, 1994; Poklis et al, 1982; Preskorn,

1997; Troy et al, 1994; van Harten, 1995; Worm et al, 1998) (see Table 8.1).

Table 8.1. Maximum recommended doses and postmortem blood or clinical plasma concentrations arising from therapeutic use of the target psychiatric drugs.

Target Drug	Maximum Dose (mg/day)[1]	Postmortem Concentrations in Whole Blood (mg/L)
Citalopram (Anastos et al, in press; Joffe et al, 1998; Worm et al, 1998)	60	0.03-0.58
Chlorpromazine (Baselt and Cravey, 2000; Caswell and et al, 2001)	300	0.3-1.9
Clozapine (Jann, 1991)	900	0.2-1.7
Fluoxetine (Goeringer et al, 2000)	80	<0.05-0.9
Fluvoxamine (Kunsman et al, 1999; van Harten, 1995)	300	0.19-2.2
Nefazodone (Baselt and Cravey, 2000; Greene and Barbhaiya, 1997)	600	0.2-2.3
Olanzapine (Baselt and Cravey, 2000; Caswell and et al, 2001)	20	0.005-0.08
Paroxetine (Goeringer et al, 2000)	60	<0.05-0.55
Risperidone (Grant and Finton, 1994; Lee et al, 1997)	8	0.003-0.008
Sertraline (Goeringer et al, 2000; Gupta and Dziurdy, 1994; Levine et al, 1994; Logan et al, 1994)	200	0.22-0.90
Thioridazine (Baselt et al, 1978; Poklis et al, 1982)	800	0.6-3.6
Trifluoperazine (Jann et al, 1985)	20	<0.05
Venlafaxine (Budd and Anderson, 1996; Jaffe et al, 1999; Troy et al, 1994)	375	0.13-0.65

[1] (Caswell et al, 2001).

Other drugs detected in combination with the targeted psychiatric drugs were similarly compared to data from clinical studies, shown in Table 8.2. It is important to note that, in general, therapeutic drug concentrations detected in postmortem blood are higher than the accepted range used for therapeutic drug monitoring in living people. This is due mainly to postmortem redistribution of these drugs, as discussed in chapter 7 and section 1.7.

Drugs detected in each case were scored for their likely contribution to death, based on information gleaned from the case examination outlined in 8.2.1 and using the following scoring system:

1 = detected at a therapeutic concentration and not likely to have contributed to death

2 = detected at a therapeutic concentration but its serotonergic activity may have enhanced effects of other drugs present

3 = detected at a supratherapeutic concentration but not likely to have contributed to death

4 = detected at a supratherapeutic concentration and likely to have contributed to death

5 = not clear whether drug contributed to death

This was adapted from that agreed to by a meeting of Australasian Senior Toxicologists for the development of a national coroners' database of toxicological information.

Table 8.2. Maximum recommended doses and corresponding clinical plasma or postmortem blood concentrations of other drugs detected in the presence of target psychiatric drugs[1,2].

Drug	Maximum Daily Dose[1]	Concentrations
Alprazolam (Fitzgerald, 1994)	10	0.03-0.7
Amitriptyline (Goeringer et al, 2000)	300	0.08-0.8
Benztropine (Jindal et al, 1981)	6	0.08-0.13
Codeine (Levine and Caplan, 1987)	360	0.07-2.0
Diazepam (Abernethy et al, 1983; Dawling and Ward, 1987)	40	0.13-0.75
Dothiepin (Crampton et al, 1980)	200	0.02-0.42
Doxepin (Ngo, 1993; Norman et al, 1980)	300	0.005-0.12
Doxylamine (Friedman and Greenblatt, 1985)	100	0.15-0.9
Gabapentin (Blum et al, 1994)	3600	2.2-8.9
Gliclazide (Malhotra, 1993)	320	≤35

Drug	Maximum Daily Dose[1]	Concentrations
Nitrazepam (Drummer, 2001)	20	0.07-0.11
Oxazepam (Knowles and Ruelius, 1972)	120	0.18-1.4
Oxycodone (Leow et al, 1992)	400	<0.1-0.42
Paracetamol (Prescott et al, 1968)	4000	15-50
Pethidine (Drummer et al, 1994)	1200	0.2-0.8
Phentermine (Groenewoud et al, 1993)	40	0.18-0.51
Procyclidine (Missen et al, 1978)		0.15-0.63
Propranolol (Walle et al, 1978)	320	0.05-0.34
Pseudoephedrine (Bye et al, 1975)	240	0.07-0.64
Salicylic acid (Hardman and Limbird, 1996)	3900	30-100

Drug	Maximum Daily Dose[1]	Concentrations
Imipramine (Nagy and Johansson, 1975)	150	0.01-0.11
Indomethacin (Bannwarth et al, 1990)	200	0.1-2.60
Metoclopramide (Goeringer et al, 2000; Saller et al, 1985)	40	0.22-1.5
Naproxen (Weber et al, 1981)	1375	31-46
Temazepam (Anastos et al, in press)	30	0.5-1.1
Theophylline (Mitenko and Ogilvie, 1973)	875	10-15
Tranylcypromine (Bailey and Barron, 1980)	30	0.08-0.1
Valproic acid (Mihaly et al, 1979)	2500	80-100

[1] Where available, therapeutic postmortem concentrations in blood are listed. In all other cases, the range of maximum plasma concentrations observed during therapeutic use is listed. [2] (Caswell et al, 2001).

In drug-related deaths, all ethanol concentrations > 0.10 g/100 mL were assigned scores of "4". Morphine, methadone, and methamphetamine, none of which are associated with a defined safe blood concentration, were also assigned "4". Drugs detected at concentrations within the accepted therapeutic range were scored as "1" if they had no known serotonergic activity, and "2" if they possessed such activity. By inference, drugs detected at trace concentrations were not expected to make a significant contribution to death and were scored as "1". A score of "3" was assigned to drugs detected at concentrations which were higher than maximum concentrations detected following therapeutic use but were unlikely to have contributed to death. Finally, a score of "5" was assigned to drugs in cases where their role in contributing to death was unclear, due to the presence of multiple other drugs at elevated concentrations and their potential for interacting with other drugs is not well established. The number of cases positive for each target drug thus receiving contribution scores of "2" or "4" were aggregated and compared.

8.2.3. Statistical analyses

Statistical evaluation of the data was performed using SPSS V9.01 for Windows software on an IBM personal computer. Paired-sample T-tests at the 95% confidence interval were used to determine whether differences in blood concentrations were statistically significant. To enable such comparison, parent drug and metabolite concentrations for each category of target drug (SSRI, mixed-reuptake inhibitor or MRI, and antipsychotics) were averaged in each type of death.

8.3. Results

The ranges and means of the subjects' ages in each death type are shown in Table 8.3. There was a statistically significant difference in the mean ages of subjects in drug-related deaths compared to natural deaths ($p<0.001$) and accidents ($p<0.05$). A gender imbalance was also observed, with almost twice as many females as males represented in natural deaths and almost three times as many females as males in drug-related deaths. Conversely, twice as many males as females died of non-drug accidents and suicides. In this study, there were no cases in which toxicity to a single target drug was given as the sole cause of a drug-related death.

Table 8.3. Means, medians, and ranges of ages of subjects in each type of death.

Death Type	Mean ± SD	Median	Range	Gender ratio (M:F)
Natural deaths (n=11)	62± 13	64	38-77	0.57
Non-drug accidents (n=6)	60 ± 15	61	41-78	2.0
Non-drug related suicides (n=27)	45 ± 14	44	18-73	2.0
Drug-related deaths (n=27)	39 ± 12	38	21-66	0.40

Non-drug deaths that tested positive for the target psychiatric drugs were categorised as either natural deaths (n=11), accidents (n=6), or non-drug suicides (n=27) (see Table 8.4).

Table 8.4. Causes of death, demographics and toxicological findings in non-drug deaths positive for target drugs [1-3] .

Case No	Cause of Death	Gender & Age	Serotonergic Drug Conc	Other Drugs
			Natural deaths	
1	Pulmonary thromboembolism	F 76	fluoxetine <0.05 (0.12)	no other drugs
2	Coronary artery thrombosis, ischemic heart disease	M 61	fluoxetine 0.09 (0.18) sertraline 0.14 (0.22)	no other drugs
3	Cardiomegaly	M 38	fluoxetine 0.30 (<0.05) risperidone 0.30 (0.40) diazepam 0.3 (0.2)	no other drugs
4	Ischaemic heart disease	M 57	paroxetine 0.10	Δ^9-tetrahydrocannabinol 6 ng/mL, propopoxyphene ~0.5, codeine (free, blood) trace
5	Subarachnoid haemorrhage 2° to ruptured berry aneurism	F 51	sertraline <0.05 (<0.05)	no other drugs
6	Acute myocardial infarct, coronary artery thrombosis, coronary artery atherosclerosis	F 66	sertraline <0.05 (0.34) risperidone 0.22 (0.25)	no other drugs
7	Bilateral pulmonary artery thromboembolism, deep vein thrombosis	F 74	sertraline ~0.1 (<0.05)	no other drugs
8	Cardiomegaly, aortic sclerosis w/ coronary artery atherosclerosis	F 73	nefazodone 1.1	no other drugs
9	Ruptured myocardial infarct, coronary artery atherosclerosis	F 77	venlafaxine 0.19 (0.08)	no other drugs

Case No	Cause of Death	Gender & Age	Serotonergic Drug Conc	Other Drugs
10	Faecal peritonitis, perforated colonic adenocarcinoma	M 64	risperidone 0.03 (<0.02)	no further toxicology
11	Pulmonary thromboembolism	F 44	risperidone 0.07 (0.26)	no further toxicology
Non-drug accidents				
12	Subdural haemorrhage	F 41	citalopram 0.14	ethanol 0.12 g/dl
13	Acute GI haemorrhage	M 45	citalopram 0.15 sertraline 0.99 (1.3)	ethanol <0.01 g/dl
14	Respiratory failure/hypoxic brain injury	M 78	venlafaxine 1.1 (1.4)	no further toxicology
15	Extensive blood loss 2° to recent arm operation	M 64	paroxetine 0.73	no other drugs
16	Head injury	M 74	paroxetine 2.7	no other drugs
17	Hepatic steatosis	F 58	sertraline 0.83 (<0.05) diazepam trace (~0.05)	ethanol <0.01 g/dl, quinine ~3.0
Non-drug suicides				
18	Multiple injuries 2° to fall	M 57	citalopram 0.39	no further toxicology
19	Hanging	M 30	citalopram 0.90 diazepam ~0.4 (~0.3) temazepam detected	codeine (free, blood) trace, (total, urine) ~11, morphine (total, urine) 0.8
20	Hanging	M 44	fluoxetine 0.07 (0.49)	ethanol 0.18 g/dl, cannabis detected
21	Hanging	M 62	fluoxetine 0.08 (0.08)	no other drugs

Case No	Cause of Death	Gender & Age	Serotonergic Drug Conc	Other Drugs
22	Carbon monoxide poisoning	F 55	fluoxetine ~0.4 (0.80)	ethanol 0.19 g/dl, carboxyhemoglobin 80 %
23	GSW to head	M 51	fluoxetine 0.76 (0.16)	ethanol (0.16 g/dl)
24	Hanging	M 43	fluoxetine 0.87 (0.39) 7-aminoflunitrazepam 0.05	no other drugs
25	Hanging	F 61	fluvoxamine 0.75 alprazolam <0.05	no other drugs
26	Hanging	M 36	paroxetine <0.05	cannabinoids detected
27	Drowning or mixed drug toxicity	F 62	paroxetine 0.60 alprazolam 0.1	propranolol 1.5
28	Hanging	M 61	paroxetine 1.1 temazepam 0.7	no other drugs
29	Carbon monoxide poisoning	M 38	paroxetine 4.8	carboxyhemoglobin 84 %
30	Multiple injuries to head and chest	F 52	sertraline <0.05 (<0.05)	no other drugs
31	Hanging	M 45	sertraline ~0.05 (0.38)	no other drugs
32	Carbon monoxide poisoning	M 40	sertraline 0.32 (0.18)	ethanol 0.02 g/dl, carboxyhemoglobin 77 %
33	Hanging	M 18	sertraline 0.35 (2.0)	no other drugs
34	Drowning	F 19	sertraline 0.46 (0.56) temazepam 0.3 olanzapine 0.3	no other drugs
35	Multiple injuries	F 54	sertraline 2.7 (5.6)	no other drugs
36	Carbon monoxide poisoning	M 39	sertraline 1.3 (<0.05)	ethanol 0.01 g/dl, carboxyhemoglobin ~76 %
37	Carbon monoxide poisoning	M 44	sertraline 1.6 (1.5)	ethanol 0.18 g/dl, carboxyhemoglobin ~78 %

Case No	Cause of Death	Gender & Age	Serotonergic Drug Conc	Other Drugs
38	Hanging	F 56	**nefazodone 4.7** alprazolam ~0.03	no other drugs
39	Multisystem organ failure in attempted hanging	F 34	**venlafaxine 0.07 (0.67)**	no other drugs
40	Carbon monoxide poisoning	M 28	**venlafaxine 0.24 (3.4)** temazepam ~0.6	ethanol (0.07), carboxyhemoglobin: ~84 %
41	Hanging	M 73	**venlafaxine 0.85 (0.52)**	no other drugs
42	Carbon monoxide poisoning	F 38	**nefazodone 0.30** **venlafaxine 1.8 (<0.05)**	carboxyhemoglobin 82 %
43	Multiple injuries due to fall, drowning 2° to venlafaxine toxicity	M 31	**venlafaxine 3.2 (1.6)** clonazepam <0.05 (<0.05)	no other drugs
44	Hanging	M 40	**olanzapine 0.1** **risperidone 0.22 (0.25)**	no other drugs

[1] All concentrations expressed in mg/L, unless otherwise stated, metabolite concentrations in parentheses. [2] Blood concentrations, unless otherwise stated. 6-AM=6-acetyl morphine. F=female, M=male. [3] Target drugs listed in boldface.

For the sake of simplicity, deaths ruled by the coroner as being heroin-related were not included in the case analyses for drug deaths, resulting in the elimination of five cases (all male subjects). However, a few cases in which morphine and other opioids were detected (although death was not attributed solely to their presence) were included.

Target drugs appear to have played at least a minor or indirect role in causing death due to their serotonergic activity (enhancing the effects of other drugs present) in all but one of the remaining 27 drug-related deaths. In 19 of these cases, target drugs were detected at supratherapeutic concentrations and therefore considered likely to have contributed to death. Table 8.5 summarises demographic and toxicological data for these 27 drug-related fatalities. A significant finding from these results is that drug combinations explicitly contraindicated in the prescribing information involving the target psychiatric drugs were detected in approximately two-thirds of the drug-related deaths (68 %). A discussion of circumstances in each type of death and prevalence of target psychiatric drugs follows.

Table 8.5. Demographics and toxicology in drug-related deaths positive for target drugs[1-4].

Case No[5]	Gender, Age	Serotonergic Drug Conc	Contribution Score	Miscellaneous Drug Concs	Contribution Score
		Cases involving a combination of target drugs			
45 (S)	M 30	citalopram 1.6 clozapine 3 (detected)	4 4	no other drugs	N/A
46 (A)	F 44	citalopram 3.1 chlorpromazine ~2.6 valproic acid 36 7-aminonitrazepam detected (trace)	4 4 2	codeine (free, blood) 0.7 morphine (free, blood) 0.05 paracetamol ~12 gliclazide ~0.7 metoclopramide detected	2 4 1 1 1
47 (A)	F 48	fluoxetine 0.39 (0.22) nefazodone 0.13 thioridazine 2.5 (meso 0.9, sulfo 0.1) alprazolam 0.06	2 2 4 2	no other drugs	N/A
48 (A)	F 61	paroxetine 14 chlorpromazine ~0.7 trifluoperazine 0.3 diazepam ~0.2 (~0.5) valproic acid detected (trace)	4 2 4 2 1	procyclidine 0.8	3
49 (A)	F 32	sertraline <0.05 (0.06) citalopram 0.2 nitrazepam 0.03 (0.4) oxazepam 0.3	2 2 2 2	naproxen 14 oxycodone 0.3 methamphetamine 0.1	1 4 4

Case No[5]	Gender, Age	Serotonergic Drug Conc	Contribution Score	Miscellaneous Drug Concs	Contribution Score
50 (A)	F 28	risperidone 0.09 (0.02)	2	ethanol 0.18 g/dl	4
		sertraline 0.2	2	morphine 0.6 (urine)	4
		diazepam 0.1 (0.1)	2	6-AM (urine)	
				codeine 0.1	1
51 (A)	F 28	risperidone 0.12 (0.12)	2	methadone 0.2	4
		thioridazine 1.2	2	morphine blood 0.5	4
		diazepam 0.1 (0.2)	2		

Cases involving target drugs in combination with MAOIs

Case No[5]	Gender, Age	Serotonergic Drug Conc	Contribution Score	Miscellaneous Drug Concs	Contribution Score
52 (S)	F 37	fluoxetine 0.29 (0.18)	2	codeine (free, blood) 0.9	3
		nefazodone 2.8	4	morphine (free, blood) trace	1
		tranylcypromine 2.3	4	paracetamol ~60	3
		temazepam 0.2	2		
		nitrazepam (stomach) ~12 mg			
		7-aminonitrazepam 0.2	2		
53 (S)	F 49	risperidone 0.10 (0.09)	2	benztropine 0.1	1
		thioridazine 5.6	4	codeine 0.19	1
		tranylcypromine 0.9	4		
		imipramine 0.4	2		
		diazepam 0.38 (0.20)	2		
54 (S)	F 42	venlafaxine 7.2 (0.7)	4	pseudoephedrine 5.8	4
		moclobemide 88	4	salicylate 40	1

Case No[5]	Gender, Age	Serotonergic Drug Conc	Contribution Score	Miscellaneous Drug Concs	Contribution Score
55 (A)	M 34	**fluoxetine 0.30 (<0.05)**	2	cannabinoids trace	1
		venlafaxine 1.8 (<0.05)	4		
		moclobemide 31	4		
		temazepam 0.6	2		
		diazepam 0.2 (0.1)	2		
56 (A)	F 37	**sertraline 1.0 (<0.05)**	4	ethanol 0.13 g/dl	4
		moclobemide 15	4		
		oxazepam 0.05	2		

Cases involving target drugs in combination with other types of serotonin-active drugs

Case No[5]	Gender, Age	Serotonergic Drug Conc	Contribution Score	Miscellaneous Drug Concs	Contribution Score
57 (A)	F 38	**paroxetine 2.9**	4	ethanol 0.17 g/dl	4
		thioridazine ~0.5	2	morphine (free, blood) 0.4 (total, urine) <0.3	4
		doxepin 0.4 (0.5)	2	6-AM (urine) 0.08	
		diazepam ~0.3 (~0.6)	2	propranolol 0.2	1
58 (S)	M 39	**venlafaxine 0.12 (1.7)**	2	phentermine 0.1	1
		doxepin ~18 (1.8)	4		
59 (S)	F 33	**venlafaxine 36 (3.5)**	4	codeine (free, blood) 0.4 (total, urine) 7.2	2
		dothiepin ~0.6	2	paracetamol ~20	1
				metoclopramide detected	1
60 (A)	F 50	**paroxetine <0.05**	2	pethidine 0.6	2
		gabapentin ~17	4	morphine (free, blood) 0.1	4
		valproic acid 49	2	codeine (free, blood) 0.05	1
		amitriptyline 0.2 (1.1)	2	doxylamine 0.1	1

Case No[5]	Gender, Age	Serotonergic Drug Conc	Contribution Score	Miscellaneous Drug Concs	Contribution Score
61 (A)	F 53	**nefazodone 0.23**	2	morphine (free, blood) 0.3	4
62 (A)	M 30	**venlafaxine 0.19 (0.76)**	2	codeine (total, urine) 0.4	1
		valproic acid (serum) 818	4	morphine (total, urine) <0.3	4
		diazepam ~0.1 (~0.3)	2	6-AM (urine) <0.02	1
63 (A)	M 32	**fluvoxamine 2.1**	4	morphine (free, blood) 0.08 (total, urine) ~30	4
		diazepam ~0.5 (~0.8)	2	codeine (free, blood) 0.04 (total, urine) 7.0	1
				theophylline trace	1
64 (S)	F 48	**venlafaxine 6.6 (2.8)**	4	codeine (free) 2.0	4
		diazepam ~0.6 (0.5)	2	morphine (free) 0.2	4
				paracetamol ~240	5
65 (A)	M 66	**venlafaxine 0.90 (0.36)**	4	methadone (1.7)	4
		nitrazepam 0.06 (0.2)	2		
66 (A)	F 40	**venlafaxine 1.8 (0.05)**	4	ethanol 0.3 g/dl	4
		diazepam ~0.3 (~1.2)	2	gliclazide ~3	2
				paracetamol ~2	1
67 (S)	M 43	**sertraline 0.31 (<0.05)**	2	ethanol 0.21 g/dl	4
		temazepam 1.0	2		
		diazepam ~0.7	2		
68 (S)	F 29	**paroxetine 11**	4	ethanol 0.45 g/dl	4
69 (S)	F 34	**venlafaxine 33 (2.1)**	4	ethanol 0.29 g/dl	4

Case No[5]	Gender, Age	Serotonergic Drug Conc[4]	Contribution Score[3]	Miscellaneous Drug Concs	Contribution Score[3]
70 (S)	F 56	risperidone <0.02 (0.02)	2	ethanol 0.19 g/dl	4
				paracetamol 100	3
				codeine 0.13	1
				doxylamine 0.1	1
				indomethacin 2	1
				procyclidine detected (trace)	1
71 (A)	F 66	sertraline 31 (2.1)	4	paracetamol ~90	4
				salicylate ~50	4

[1] All concentrations expressed in mg/L, unless otherwise stated; metabolite concentrations in parentheses. [2] Blood concentrations, unless otherwise stated. 6-AM=6-acetyl morphine. F=female, M=male. [3] Contribution scores: 1 = therapeutic, not likely to contribute to death; 2 = therapeutic, serotonergic activity may have contributed to death; 3 = supratherapeutic, not likely to have contributed to death; 4 = supratherapeutic, likely to have contributed to death; 5 = contribution unclear. [4] Target drugs listed in boldface. [5] (A): accident, (S): suicide.

8.3.1. Non-drug deaths

8.3.1.1. Natural deaths

As expected based on the contribution scoring criteria outlined in section 8.2.2, there were no cases ruled as natural deaths in which the target drugs were considered to have played a contributory role, i.e. all concentrations were in the accepted normal therapeutic range. Deaths in these cases largely involved exacerbated chronic heart conditions, pulmonary or coronary artery thromboses, or acute myocardial infarcts. The subjects who died of natural causes expectedly comprised the oldest age group (mean age: 62 ± 13 years). There were more females than males in this group of subjects (7 females versus 4 males).

8.3.1.2. Accidents and traumatic deaths

Three of the non-drug accidents and traumatic deaths resulted from haemorrhaging or blood loss associated with complications of recent operations or injuries. Few substances other than ethanol were detected in addition to the target drugs. The mean age of subjects in this group (60 ± 15 years) was 2 years less than that of subjects who died of natural causes. There were twice as many males as females (8 males versus 4 females).

Out of the deaths in this group, only paroxetine (case 16) is likely to have contributed to the mode of death. In this case, a 74-year-old male with a history of accidental falls stumbled while

attempting to climb the stairs in his home. He struck his head on the floor, suffered a seizure, and lost consciousness. He was taken to hospital but never regained consciousness. Autopsy findings included cirrhosis of the liver, chronic obstructive airways disease, and idiopathic Parkinson's disease. Toxicological testing showed the presence of paroxetine only (2.7 mg/L) (Table 8.4). It is possible that the paroxetine concentration was elevated due to misuse or possibly because his cirrhosis resulted in decreased drug clearance. Although death in this case was clearly attributable to his physical injuries, the paroxetine concentration may have affected his behaviour, making him dizzy or unsteady on his feet and ultimately contributing to his fatal fall.

8.3.1.3. Non-drug suicides

Death by hanging (n=12) was the most commonly employed method of suicide in this class, followed by carbon monoxide poisoning (n=7). The mean age of the subjects in this group (45 ± 14 years) was much lower than that of the subjects in either the natural deaths (27 %) or accidents (25 %) (see Table 8.3). There were twice as many males as females who died of accidents (18 males versus 9 females). There were two non-drug suicides in which one or more target psychiatric drugs were detected at elevated concentrations (cases 29 and 38).

In case 29, a 38 year-old male with a history of depression and suicide ideation was found dead in his car with a hose running from the tailpipe into the rear window. Two opened packets of Aropax® were found in the glove box. Evidence of recent heavy drinking was found in his home. No suicide note was found. At autopsy, the subject was found to have cherry red lividity, patchy

myocardial fibrosis, pulmonary oedema, and fatty liver. No alcohol was detected in the subject's blood. Toxicology revealed the presence of no drugs other than paroxetine (4.8 mg/L) (Table 8.4). Given the substantially elevated concentration, it is likely he took a number of paroxetine tablets in his suicide attempt prior to succumbing to carbon monoxide poisoning.

Case 38 involved the death of a 56 year-old female with a history of bi-polar disorder, anorexia nervosa, and suicide ideation who had repeatedly suffered domestic violence at the hands of her husband. Her husband found her hanging from the stairwell at their home and unsuccessfully attempted CPR. At autopsy, dry brown indented abrasions around her neck were noted. In addition to nefazodone (4.7 mg/L), alprazolam was detected in the subject's blood at a concentration of approximately 0.3 mg/L (Table 8.4). Like the subject in case 29, the subject in case 38 is likely to have taken an excess of nefazodone prior to hanging herself.

8.3.2. Drug-related deaths

The mean age of the subjects in drug-related deaths (38 ± 9 years) was significantly lower than that of the subjects in any of the three types of non-drug death already discussed (see Table 8.3). There were many more females than males represented in this category of death (8 males versus 20 females). The cause of death in 19 of these cases were considered to have been at least partially attributable to one or more of the target psychiatric drugs (see Table 8.5).

Review of the circumstances in these cases reveals that mixed-drug toxicities involving the target psychiatric drugs can be divided into three categories: a) deaths involving two or more target

drugs in combination, b) deaths in which target drugs were detected in combination with an

MAOI, and c) deaths in which target drug was involved in combination with other selected drugs

with known serotonergic activity.

8.3.2.1. Deaths involving a combination of one or more target drugs

Accidental toxicity primarily due to one or more target drugs appears to have occurred in seven

subjects (cases 45-51). In cases 45-48, target drugs were deemed to have played a major role in

causing death. Death in cases 45 and 48 may have arisen due to a target drug causing bleeding

disorders stemming from decreased platelet serotonin concentrations.

Case 45 involved a 30 year-old male with a history of depression, schizophrenia, and previous

suicide attempts who was found dead on the floor of his home. A small amount of blood

emanating from the corner of his mouth was noted at the scene. The only significant finding at

autopsy was a fatty change of the liver. Toxicological testing showed the presence of citalopram

and clozapine (Table 8.5). The elevated concentrations of these two drugs could have caused

serotonin syndrome in this subject, although no information was available regarding symptoms to

confirm any clinical changes.

In case 48, a 61 year-old female with a history of intellectual disability, behavioural disturbances,

dementia, pseudoparkinsonism, scoliosis, multiple injuries due to a recent fall, and a previous

myocardial infarct was found dead in bed with epistaxis from a bleeding nasal polyp. Three days

prior to her death she had been treated for haematemesis. Autopsy showed moderate pulmonary

oedema and congestion, patchy ventricular fibrosis, nephrosclerosis, ulcers on both feet, and melanosis coli. Her left elbow had been set at 90 degrees to the rest of her body to heal. Toxicological testing revealed the presence of paroxetine, chlorpromazine, and trifluoperazine (Table 8.5). Cause of death in this case was combined drug toxicity with a contributing factor of chronic obstructive airways disease. However, like earlier cases, the presence of multiple serotonin-active drugs in this case increases the likelihood that this subject developed a hyper-serotonergic state prior to her death.

In case 46, a 44 year-old female with a history of asthma, obesity, diabetes, lymphoma, fear of death, and depression was found face down on the floor. Ambulance officers attended but were unable to resuscitate her. In addition to confirming the diagnosis of lymphoma, autopsy findings included cardiomegaly, pulmonary oedema, and a dilated right ventricle. Toxicological testing revealed elevated citalopram and chlorpromazine concentrations, as well as valproic acid (detected at therapeutic concentrations) and many other drugs (Table 8.5). Death in this case was ascribed as primarily due to citalopram, although the combined serotonergic activity of chlorpromazine and valproic acid may have contributed to toxicity. The supratherapeutic concentrations of target drugs detected in these cases had the potential to cause serotonin syndrome.

In contrast to case 46, death in case 47 was attributed by the coroner primarily to an elevated target antipsychotic concentration (thioridazine) in the presence of fluoxetine and nefazodone (both detected at therapeutic concentrations). The 48 year-old female in this case had a history of depression and drug abuse, in addition to a family history of heart failure was found dead at

home. Autopsy revealed hepatomegaly, hepatosteatosis, congestive splenomegaly, and diverticulitis. In addition to the target drugs and metabolites, alprazolam was detected at a therapeutic concentration, which may have enhanced the serotonergic activity of the other drugs (Table 8.5). Although this subject died alone, circumstances in this case strongly support the possibility that the combination of detected drugs led to a hyper-serotonergic state, ultimately resulting in death.

In cases 49-51, target drugs (sertraline, citalopram, and risperidone, and thioridazine) were detected at therapeutic concentrations, death in each case being attributed primarily to the presence of an opioid (oxycodone, methadone, and morphine). Diazepam was also detected in cases 49 and 51 (Table 8.5). However, it is possible their serotonergic activity enhanced the effects of other detected drugs. These cases involved 28-32 year-old females with histories of depression and drug abuse who were found dead at home. Cardiomegaly was observed at autopsy in case 49. Autopsy findings in cases 50 and 51 showed the presence of needle puncture marks in the cubital fossa as well as pulmonary oedema (in case 51). In each of these cases, although target drugs were detected at therapeutic concentrations, their combined presence could have acted synergistically to cause serotonin syndrome.

8.3.2.2. Deaths involving target drugs in combination with MAOIs

In cases 52-56, a monoamine oxidase inhibitor (MAOI) was detected in the presence of at least one target drug. Cases 52-54 all involved females (37, 49, and 42 years old, respectively) with histories of depression and suicide ideation (including previous suicide attempts in case 53) who

died following multiple-drug overdoses. Subject 52 was reported missing from the mental health clinic after being given unsupervised day leave. In cases 52 and 54, no significant anatomical findings were revealed at autopsy. In case 53, the presence of cardiomegaly, a congested myocardium, fatty replacement of the ventricular wall, fatty degeneration of the liver, adrenal cortical adenoma, pulmonary oedema, follicular adenoma of the thyroid, and aspirated gastric material were observed. In each of these cases, an MAOI (either tranylcypromine or moclobemide) and at least one target drug (nefazodone, thioridazine, and venlafaxine in cases 52, 53, and 54, respectively) was present at supratherapeutic concentrations and therefore likely to have contributed to death. Both codeine and the detected benzodiazepines may also have contributed to death in these cases. The presence of an SSRI, MRI, or serotonin-active antipsychotic in these cases in combination with an MAOI is specifically contra-indicated in the prescribing information due to the propensity for patients to develop serotonin syndrome.

Cases 55 and 56 were primarily ascribed to accidental intoxication to an MAOI (tranylcypromine and moclobemide, respectively), although target drugs (nefazodone and sertraline) were also detected at concentrations sufficient to be considered to play at least a partial role in death (Table 8.5). In case 55, fluoxetine was also detected at therapeutic concentrations but its pharmacological activity may have contributed to serotonergic excess. The same is true for the presence of benzodiazepines in both cases.

Case 55 involved a 34 year-old male with a history of suicide attempts and violence towards his wife who was taken to hospital after being observed to have taken sleeping pills. There he grew "hypertensive and increasingly stiff" during treatment, and eventually died. The only significant

autopsy finding was bronchopneumonia. The combination of drugs as well as the circumstances of the case suggest the development of serotonin syndrome led to this subject's death.

The 37 year-old female in case 56, who had a history of a brain tumour, recent brain surgery, muscular dystrophy, and depression, was found dead in bed. Ambulance officers attended the scene but were unable to resuscitate her. A bottle containing 50 mL of champagne was found on the dresser. Autopsy findings confirmed her recent brain surgery. Like other subjects already discussed, the fact that the subject in this case died alone makes it difficult to determine the mechanism that caused death. However, since both moclobemide and sertraline were detected at elevated concentrations, the subject could have developed serotonin syndrome that resulted in death. The oxazepam present may also have played a contributory role.

8.3.2.3. Deaths involving a target drug in combination with other selected drugs with known serotonergic activity

Deaths in cases 58-62 were all attributed to toxicity to one or more target drugs in combination with other types of drugs. Elevated concentrations of serotonergic drugs were detected in two cases (subjects 58 and 59). Pathological findings in case 61 indicate the subject developed serotonin syndrome prior to death.

Cases 58 and 59 involved the death of subjects in their 30's (one male and one female) with histories of depression and relationship trouble who were found dead at home. Each subject had venlafaxine in combination with a tricyclic antidepressant (TCA), as well as other drugs in their blood, including phentermine (in subject 58), paracetamol and codeine (in subject 59) (Table

8.5). In case 58, death was primarily caused by doxepin, whereas venlafaxine was the primary contributor in case 59. Pulmonary oedema was observed in both cases at autopsy, in addition to evidence of fatty liver, a colloid cyst in the brain (3rd ventricle), and splenomegaly in case 58. The elevated concentrations of doxepin in case 58 and of venlafaxine in case 59 were sufficient to have caused death alone. However, it is also possible that both subjects developed serotonin syndrome prior to death.

Although the nefazodone concentration in case 61 was within the accepted therapeutic range (see Table 8.1), the case circumstances are important to mention as the pathologist ruled the death was caused by serotonin syndrome. The case involved the death of a 53 year-old female with a history of depression and alcoholism (although no ethanol was detected). She was found at home, face down, about one or two days after she died. Autopsy revealed severe renal failure and granular material within the tubules (thought by the pathologist to be myoglobin), which are suggestive of recent malignant hyperthermia. Toxicological testing of blood also showed morphine (0.3 mg/L) (Table 8.5). Impaired hepatic function resulting from the subject's chronic alcoholism may have led to an inability to clear the drug, thus creating a setting in which a normally therapeutic nefazodone concentration caused serotonergic excess.

The subjects in cases 60 and 62 both were taking antiepileptic medications in combination with target psychiatric drugs and pain medication. However, they do not share many other similarities in circumstances.

Case 60 involved a 50 year-old female with a history of migraine headaches but no epileptic seizures. She had been enrolled in a clinical trial for gabapentin to treat her headaches and who was found dead in bed. Autopsy showed the presence of coronary artery disease, cardiomegaly, pulmonary oedema, a right fibrial cyst, previous cholecystectomy, fatty liver, and microscopic changes in lungs consistent with asthma. Paroxetine, potentially fatal concentrations of pethidine, amitriptyline and gabapentin, as well as morphine and valproic acid were detected in toxicological testing. The pathologist who performed this autopsy did not rule out the possibility that the death was due to drug toxicity. He left the final decision to the coroner, who ruled the death as being due to coronary artery disease (Table 8.5). However, the circumstances of this case provide strong evidence for the death being due to drug toxicity, with the subject's coronary artery disease being a contributing factor.

Case 62 involved the death of a 30 year-old male with a history of depression and suicide attempts who suffered a seizure after being sent home from hospital after his last overdose attempt. He was observed to recover from "the shakes" and later died before the morning. Empty packets of a variety of medications were found at the scene. Autopsy findings included evidence of HIV and hepatitis C, pulmonary oedema and congestion, and haemorrhage of the upper gastrointestinal tract. Toxicological testing showed the presence of an elevated valproic acid concentration in serum, as well as venlafaxine, O-desmethylvenlafaxine and diazepam in the subject's blood (Table 8.5). Codeine and morphine were also detected in urine. Although primarily a valproate death, the extremely elevated valproic acid concentration in combination with venlafaxine, as well as the development of seizures make it difficult to rule out contribution from serotonin syndrome.

8.3.3. *Indicators of relative target drug toxicity*

There was some overlap in parent drug and metabolite concentrations between each category of death (see Table 8.6). However, SSRI concentrations detected in natural deaths were significantly lower (88 %) than those measured in non-drug suicides ($p < 0.05$). No other significant differences in target drug concentrations were observed between any other categories or any other drug types.

Table 8.6. Drug concentrations of psychiatric drugs in blood from 71 postmortem cases[1,2].

Type of Death	SSRIs	SSRI mets	MRIs	MRI mets	Antipsychs	Antipsych mets
Natural (n=11)	0.11 ± 0.08 (<0.05-0.30)	0.14 ± 0.11 (<0.05-0.34)	0.60 ± 0.71 (0.19-1.1)	0.08 (0.08)	0.16 ± 0.13 (0.03-0.30)	0.23 ± 0.16 (<0.02-0.40)
Non-drug Accidents (n=6)	0.92 ± 0.94 (0.14-2.7)	0.68 ± 0.88 (<0.05-1.3)	1.1 (1.1)	1.4 (1.4)	no cases	no cases
Non-drug Suicides (n=27)	0.88 ± 1.1 (<0.05-4.8)	0.94 ± 1.5 (<0.05-5.6)	1.6 ± 1.8 (0.07-3.2)	1.3 ± 1.3 (<0.05-3.4)	0.16 ± 0.08 (0.1-0.22)	0.25 (0.25)
Drug-related Deaths (n=27)	4.3 ± 8.2 (<0.05-31)	0.39 ± 0.76 (<0.05-2.1)	7.6 ± 13 (0.10-36)	1.3 ± 1.3 (<0.05-3.5)	1.4 ± 1.7 (<0.02-5.6)	0.21 ± 0.34 (0.02-0.90)

[1] All concentrations in mg/L, unless otherwise stated. Means ± SD, with ranges in parentheses.

[2] SSRIs: citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline. MRIs (mixed reuptake inhibitors): nefazodone, venlafaxine. Antipsychotics: chlorpromazine, clozapine, olanzapine, risperidone, thioridazine, trifluoperazine.

The number of drug-related deaths (either accidents or suicides) in which a target drug appears to have contributed to death are summarised in Table 8.7. It can be seen that there were 21 instances of a target drug being scored as a major contributor to death, with venlafaxine (n=7) and paroxetine (n=3) most often being implicated. However, this is to be expected, as both accounted for a large percentage of the total number of target drug prescriptions given under the Prescription Benefits Scheme in Victoria over the period of this dissertation (17 % and 22 %,

respectively). There were an additional 17 instances of a target drug being considered a minor

contributor to death, with fluoxetine, risperidone, and sertraline equally implicated in the most

number of cases (n=3 for each drug).

Table 8.7. Number of cases of drug-related deaths where target drugs played a contributory role[1].

Target Drug	Major Contributor	Minor Contributor	No of Cases
Chlorpromazine	1	1	2
Citalopram	2	1	3
Clozapine	1	0	1
Fluoxetine	0	3	3
Fluvoxamine	1	0	1
Nefazodone	1	2	3
Paroxetine	3	1	4
Risperidone	0	3	3
Sertraline	2	3	5
Thioridazine	2	2	4
Trifluoperazine	1	0	1
Venlafaxine	7	1	8

[1] Major contributor: score = 4, supratherapeutic and likely to have contributed significantly to death; minor contributor: score = 2, therapeutic but serotonergic activity may have enhanced effects of other detected drugs.

8.4. Discussion

Determining the role of the target antidepressant drugs in fatalities is difficult, as no clear correlation has been reported between plasma concentrations and clinical efficacy (Baumann, 1996). This is further complicated by the multiple use of drugs in every case, and the paucity of published data relating blood concentrations of the target drugs arising from therapeutic use in the postmortem setting. This is particularly true for nefazodone and risperidone, for which no postmortem therapeutic concentrations in blood had been published as of the time of writing. However, some trends were observed in the cases investigated in this study.

Pathological findings at autopsy related to the target psychiatric drugs were of limited use in interpreting their role in causing fatality, as the vast majority of cases involved multiple drugs. Therefore, autopsy findings were most often reflective of natural diseases commonly observed in people with histories of drug abuse or psychiatric conditions. The most common findings were liver disease (cirrhosis and fatty liver) (25 cases), agonal processes such as pulmonary oedema and congestion (21 cases), heart disease (coronary artery atherosclerosis, ischaemic heart disease, cardiomegaly, and/or myocardial infarcts) (16 cases). Pulmonary oedema is commonly seen in heroin abusers as a result of respiratory depression (Gerlach, 1980; Helpern, 1972; Jannsen and Van Bever, 1978; Karch, 1993).

Without pathological indicators, diagnosis of serotonin syndrome can only be made by observation of the circumstances and any clinical symptoms (see Table 1.11 for review). For example, muscle rigidity and hypertension in case 58 could be explained by serotonin syndrome,

although the only significant postmortem finding was bronchopneumonia. However, in one case (subject 61) there was clear evidence that death was caused by the development of serotonin syndrome. From the circumstances in the presented cases, it appears that renal/other organ failure or rhabdomyolysis (both suggestive of malignant hyperthermia) provide evidence that a particular subject developed serotonin syndrome. Elevated creatine phosphokinase concentrations (typical for neuroleptic malignant syndrome) are not typically present in serotonin syndrome. Other indicators include evidence of CNS infections, tumours, lupus, and heatstroke (Goldberg, 1998). Finally, noting the presence of drug combinations known to cause serotonin syndrome (the target drugs in particular, either alone or in combination with MAOIs, tricyclics, or other drugs with known serotonergic activity) may aid in determining the likelihood of this syndrome developing.

It is of interest that in 49 cases, serotonin-active drugs could have exacerbated pathological findings. This includes subjects noted to have cardiovascular disease (n=16), hepatic impairment (n=25), or evidence of haemorrhaging (n=8).

In four of the drug-related deaths, autopsy findings included evidence of cardiovascular disease. This is cause for concern since a number of reports of the association of SSRIs with cardiac arrhythmias and other cardiovascular complications were published approximately 10 years ago (Buff et al, 1991; Ellison et al, 1990; Feder, 1991; Gardner et al, 1991; Golino et al, 1991; Golino et al, 1994; Hillis and MacIntyre, 1993; McFadden et al, 1991; Skop et al, 1994; Spier and Frontera, 1991). Most of these reports were in regard to fluoxetine, although vasoconstriction in

patients with ischaemic heart disease has also been reported in subjects taking paroxetine (Skop et al, 1994).

Serotonin receptors modulate vascular resistance in a complex manner. In healthy people, serotonin causes vasodilatation. However, in damaged endothelium, serotonin produces direct unopposed vasoconstriction modulated by 5-HT_{1A} receptors (Golino et al, 1991). As a result, it has been theorised that an increase in myocardial ischaemia secondary to an increase in vasoconstrictive serotonin in the blood can occur in treatment for depression with serotonin-active drugs (Gardner et al, 1991; McFadden et al, 1991; Skop et al, 1994; Spier and Frontera, 1991).

It is notable that six subjects whose deaths were ascribed to natural causes had heart disease. The enhanced effect of serotonergic drugs (particularly fluoxetine, paroxetine, and sertraline) in the setting of chronic heart disease may have put these comparatively older subjects at an increased risk of death even though drug concentrations were well within the accepted therapeutic range.

The presence of a high gabapentin concentration in case 60 (17 mg/L) may have contributed to serotonin-related vasoconstriction. Plasma concentrations between 2–5 mg/L are generally accepted as therapeutic (Baselt and Cravey, 2000). The use of antiepileptic drugs in migraine treatment has been previously demonstrated (Hering and Kuritzky, 1992; Jensen et al, 1994; Lenaerts et al, 1996; Matthew et al, 1995; Pavese et al, 1994; Sorensen, 1988). The pain associated with migraine headaches originates either from neurogenic inflammation within the dural vasculature (characterised by plasma protein extravasation, vasodilatation, and mast cell

degranulation) or neurogenic vasodilatation of dural blood vessels which may be a key component of the observed inflammation (Williamson and Hargreaves, 2001). Therefore, the clinical efficacy of drugs used in migraine therapy (such as sumatriptan) is linked to their ability to cause vasoconstriction.

The mechanism of effect for antimigraine drugs is cause for concern since migraine and major depression are often co-morbid, requiring concurrent treatment (Moldin et al, 1993; Silberstein, 1998). Additionally, the development of migraines in conjunction with SSRI treatment has been reported, particularly with fluoxetine, fluvoxamine, and paroxetine (Delva et al, 2000; Larson, 1993; Szabo, 1995). However, they have also been used for migraine prophylaxis (Adley et al, 1992; Black and Sheline, 1995; Lewis and Solomon, 1995). Regardless, it appears that the combination of serotonergic antidepressants, particularly SSRIs, with antiepileptics carries a risk of increased likelihood of toxicity. It is possible that subject 60 developed serotonin syndrome, although this subject also had significant coronary artery atherosclerosis, which complicates the assessment of cause of death.

Case circumstances suggest five of the subjects whose deaths were drug-related developed bleeding disorders prior to death. The deaths of three subjects in non-drug accidents also resulted from haemorrhaging or blood loss associated with complications of recent operations or injuries. Numerous reports of the onset of bleeding complications following initiation of treatment with SSRIs can be found in the literature (Aranth and Lindberg, 1992; Fischer et al, 1995; Shapiro, 1996; Yaryura-Tobias et al, 1991). Spontaneous ecchymosis and splenomegaly may accompany the abnormal bleeding (Goldberg, 1998). These reports have focused on bleeding disorders

associated with fluoxetine, fluvoxamine, and sertraline, although they can occur with other serotonin-active drugs as well.

The mechanism through which bleeding disorders occur is related to circulating serotonin, which is stored in platelets. At sites of vascular tears, platelets release serotonin, triggering platelet aggregation and vasodilatation which allow clotting to occur without thrombosis (Goldberg, 1998). However, psychiatric drugs with serotonergic activity block reuptake of serotonin into platelets, which could impair platelet aggregation and prolong bleeding. For example, the upper gastrointestinal tract haemorrhage noted in subject 62 may have been related to changes in platelet serotonin. The likely occurrence of bleeding disorders associated with psychiatric drug therapy highlights another way in which the target drugs could indirectly contribute to death, particularly in older patient populations, even though blood concentrations are within the therapeutic ranges.

There were 13 of the 27 reported drug-related deaths in which the subject may have developed serotonin syndrome prior to death (48 %). These cases fell into three categories, as discussed in section 8.3.2: a) deaths involving a combination of more than one target drug, b) deaths involving target drugs in combination with MAOIs, and c) deaths involving other selected drugs with known serotonergic activity.

The possible development of serotonin syndrome in cases falling into categories a) and c) is interesting since eight cases in the studied group had one or more target drugs present. One example is valproic acid. Like other antiepileptic drugs, valproic acid primarily affects GABA

receptors, but it has been shown that serotonin receptors, particularly $5\text{-}HT_{2A}$ and $5\text{-}HT_{1A}$, potentiate GABA activity and that the drug achieves its pharmacological effect through a complex interaction with both serotonin and GABA (Winterer and Hermann, 2000). Further, Baf et al have shown changes in brain serotonin (as well as noradrenaline and dopamine) concentrations following administration of sodium valproate to rats (Baf et al, 1994). Specifically, observed changes in serotonin concentrations included significantly increased 5-HT concentrations in striatum-accumbens and brain stem ($p<0.001$), marginally (but not significantly) increased 5-HT concentrations in motor cortex and hippocampus, and significantly decreased 5-HT concentrations in hypothalamus ($p<0.001$) and cerebellum ($p<0.01$). The authors concluded that valproic acid's anticonvulsant effect may be a result of alterations in monoamine concentrations apart from its action on GABA.

Rarely, deaths have been reported from valproic acid alone. Fatal hepatitis was reported in three paediatric patients, one of whom developed a serum valproic acid concentration of 166 mg/L (Donat et al, 1979; Suchy et al, 1979). A teenage girl who died in a coma after intentionally overdosing on valproate was found to have a peak plasma concentration of 1969 mg/L (Garnier et al, 1982). In another case in which a man died after ingesting an estimated 50 g of valproate, blood and liver concentrations of 1050 mg/L and 985 mg/L, respectively, were detected (Poklis et al, 1998). The presence of valproate at 818 mg/L in case 62 could be sufficient to cause death in combination with the other drugs detected through the development of serotonin syndrome, particularly in light of the presence of venlafaxine and diazepam, even though these drugs were detected at therapeutic concentrations.

In five cases, moclobemide or tranylcypromine, both MAOIs, were considered to play a major role in contributing to death in combination with a target drug due to their known propensity to cause serotonin syndrome (Caswell and et al, 2001). The presence of SSRIs in combination with MAOIs has been clearly linked with the development of this syndrome due to the combined serotonergic activity of more than one drug (Singer and Jones, 1997). It has previously been recognised that adverse reactions between MAOIs and SSRIs can occur even when the SSRIs are present at normally therapeutic concentrations (Drummer, 1998; Lane and Baldwin, 1997).

The possibility of CYP450 isoenzyme metabolic inhibition is complex with respect to serotonergic drugs. Each drug or metabolite possesses not only a differing degree of pharmacological activity, but also an ability to act as a substrate or inhibitor of different P450 isoenzymes. According to two studies (Brosen, 1993; Naranjo et al, 1999), citalopram and sertraline hold less potential for interactions with other drugs that affect the CNS. Both drugs are characterized by weak inhibition of CYP450 isoenzymes compared to the other SSRIs (refer to section 1.4 for review). Paroxetine and fluoxetine potently inhibit CYP2D6, which can result in important adverse reactions with neuroleptics and antidepressants metabolised by the same enzyme (Edwards and Anderson, 1999; Sproule et al, 1997).

Fluoxetine weakly inhibits CYP3A4 and 2C19, giving rise to the possibility of interactions with benzodiazepines. Nefazodone may also carry the possibility of interactions with benzodiazepines because it weakly inhibits CYP3A4. Fluvoxamine's status as a potent inhibitor of CYP1A2, a moderate inhibitor of 3A4, and a weak inhibitor of 2D6 make it most likely to interact with clozapine and benzodiazepines. Venlafaxine inhibits both CYP2D6 and 3A4, although to a lesser

extent than the SSRIs. Clinically relevant, potentially hazardous drug interactions with SSRIs which have been reported in the literature are summarised in Table 8.8. It should be noted that lack of inclusion of particular SSRIs for some interactions does not mean the interaction will not occur with that drug, only that such an interaction has not been reported.

Out of all the listed adverse drug interactions, clinically CYP450 enzyme inhibition of 3A4 may have occurred in 17 of the presented cases in which benzodiazepines and SSRIs were detected in combination. However, competitive inhibition of 2D6 due to the combined presence of SSRIs with an MAOI is also likely, and may have occurred in four cases. Similar inhibition of 2D6 due to the combined presence of an SSRI with a TCA in two cases may have resulted in increased TCA concentrations. However, since the combination of SSRIs with either MAOIs or TCAs is known to lead to development of serotonin syndrome, it is difficult to separate out the contribution of isoenzyme inhibition.

Table 8.8. Clinically relevant, potentially hazardous drug interactions involving SSRIs[1].

SSRIs	Interacting Substance	Possible Result
All	**Anticoagulant** acenocoumarol warfarin	Possible enhanced anticoagulant effect
All	**Antidepressant** MAOIs[2] tricyclics	CNS toxicity[3] Increased plasma level of some tricyclics
Fluoxetine	**Antiarrythmic** flecainide	Increased plasma concentration of flecainide
Fluoxetine, fluvoxamine	**Anticonvulsant** carbamazepine	Lowering of convulsive threshold
All	**Antihistamine** terfenadine	Increased risk of arrythmias
All	**Antimanic** lithium	CNS toxicity[3]
All	**Antimigraine** sumatriptan	CNS toxicity[3]
Fluoxetine, fluvoxamine Fluoxetine Fluoxetine, paroxetine	**Antipsychotic** clozapine haloperidol sertindole	Possible increased plasma clozapine conc Increased plasma haloperidol concentration Increased plasma concentration of sertindole
All	**Antiviral** ritonavir	Possible increased conc of some SSRIs
Fluvoxamine	**Anxiolytic/hypnotic** benzodiazepines	Increased plasma concentration of some benzodiazepines
Fluvoxamine	**Bronchodilator** theophylline	Increased plasma theophylline concentration
Fluoxetine	**Dopaminergic** selegiline	CNS excitation, hypertension
All	**5-HT$_1$ agonist** sumatriptan	CNS toxicity[3]
All	**Opioid analgesic** tramadol	Possible increased risk of convulsions

[1] Adapted from Edwards and Anderson (Edwards and Anderson, 1999). [2] This interaction is less likely to occur with newer, reversible MAOIs. [3] CNS toxicity includes excitation, restlessness, sweating, flushing, pyrexia, fluctuating vital signs, tremor, rigidity, myoclonus, delirium, and sometimes coma and death.

In summary, the toxicity of targeted psychiatric drugs is greatly increased when taken in combination with other drugs. The most interesting finding was that almost all subjects were using more than one serotonin-active drug, which raises the possibility of either inadequate control with one drug or deliberate drug misuse. Of most concern were some potentially serious adverse drug combinations, such as multiple targeted drugs alone or in combination with MAOIs or other known significantly active serotonergic drugs. This is especially disturbing since such combinations are explicitly contra-indicated in the prescribing information for these drugs. The cause of this is unclear. It may be that subjects taking these drugs, whether "doctor-shopping" or self-medicating, are unaware of the potential complications. Out of the 27 drug-related deaths, 11 were suicides and 16 were accidental toxicities, suggesting toxicity due to one or more target drugs is more often than not unintentional. It may also be that doctors are unaware either 1) of the dangers involved with prescribing such combinations, or 2) of the patients' full medical histories which might reveal they are already taking a serotonin-active drug. Regardless, the consequences of such practices have been shown in a number of the presented cases.

Toxicity stemming from the serotonergic activity of one or more target drugs can occur due to the development of serotonin syndrome, bleeding disorders, or cardiac complications. Additionally, metabolic inhibition of one or more CYP450 isoenzymes may increase the likelihood of toxicity. This may be true even when a target drug is detected at apparently therapeutic concentrations, as in the setting of hepatic dysfunction. It is hoped these findings both aid in diagnosis of causes of death resulting from such serotonergic activity as well as highlight risk factors in the prescription of the target psychiatric drugs and ultimately will assist the toxicologist in the interpretation of the significance of these drugs in cases.

CHAPTER 9 : GENERAL DISCUSSION

9.1. Introduction

Although generally considered safer in overdose than the classical TCAs, SSRIs, atypical antidepressants, and atypical antipsychotics also cause death, yet the frequency and factors associated with such cases are less clear than in those involving the TCAs (Singer and Jones, 1997; Springfield and Bodiford, 1996; Vermeulen, 1998).

A major reason for this may be the growing practice of polypharmacy in refractory patients (Ananth and Johnson, 1992; Goldman, 2000). Additionally, persons with histories of drug abuse commonly resort to "doctor shopping" to obtain particular drugs they seek. Both practices complicate the interpretation of the role of a psychiatric drug in a death, as adverse drug interactions can occur. Such drug interactions can present either in the form of enhanced serotonergic activity due to the presence of more than one serotonin-active drug, vasoconstriction in subjects with ischaemic heart disease, bleeding complications due to decreased platelet serotonin concentrations, or delayed drug/metabolite clearance due to metabolic inhibition of a particular CYP450 isoenzyme.

Assessing the degree to which adverse drug reactions might have caused death is difficult because a detailed account of antemortem symptoms is often not available. This is particularly true in the case of serotonin syndrome (SS) and neuroleptic malignant syndrome (NMS), the diagnoses of which are both highly dependent on recognition of specific symptoms.

At the time this dissertation commenced, there was little collective data on the postmortem tissue distribution and redistribution of the target psychiatric drugs. Because of this, the ability to properly interpret toxicological data in deaths involving these drugs is currently limited.

The aim of this dissertation was to employ LC-MS methods to detect a range of psychiatric drugs and to use data obtained from coronial cases to improve the understanding of their toxicology in cases where they were a factor in death. Using a total of 107 cases, the role of psychiatric drugs in contributing to death was evaluated. The drugs selected for study in this dissertation were representative of the variety of psychiatric drugs typically detected in Coroners' cases in Australia. They included five SSRIs (citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline), two "mixed-uptake inhibitors" (nefazodone and venlafaxine) and a number of antipsychotic drugs (chlorpromazine, clozapine, flupenthixol, fluphenazine, haloperidol, olanzapine, risperidone, thioridazine, and trifluoperazine).

The research for this dissertation specifically included the following:

- development of an analytical method using LC-MS to identify and quantify the concentrations of targeted psychiatric drugs and metabolites in a number of postmortem tissues,

- development of an analytical method using LC-MS to identify and quantify the concentrations of selected drugs of abuse and their metabolites in postmortem blood,

- determination of psychiatric drug distribution patterns in postmortem tissue in deaths where the drugs had been detected in blood,

- determination of antipsychotic drug distribution in postmortem schizophrenic brain regions.

- an investigation as to whether selected psychiatric drugs and their metabolites undergo postmortem redistribution, and

- review of circumstances, pathological and toxicological findings of a series of deaths in which psychiatric drugs were detected in order to identify the types of serotonin-active drugs. These data were used to assess any possible adverse interactions.

9.2. Psychiatric drug assay development

To accurately and precisely determine the postmortem concentrations of psychiatric drugs and their active metabolites, an important part of my research was to develop an analytical technique to detect and quantitate the drugs and metabolites in a variety of postmortem specimens. Although there are a number of papers describing the chromatographic separation of small groups of the drugs and metabolites under investigation, they were limited in that either they were unable to separate polar or large molecular weight compounds or lacked the appropriate selectivity to separate and detect individual drugs in the complex mixtures commonly seen in drug related fatalities. At the time this dissertation commenced the few published methods that used LC-MS for psychiatric drug determination focused on the measurement of a small number of drugs.

A butyl chloride extraction method currently used at the Institute for routine analysis of tricyclic antidepressants using HPLC-DAD (McIntyre et al, 1993a) was modified for use with LC-MS.

This method was used in the determination of psychiatric drugs in blood, bile, brain, liver, urine, and vitreous humour. The LC-MS method used in this research enhanced the selectivity and provided higher sensitivity compared to LC-DAD methods.

The final analytical method employed the use of isocratic liquid chromatography carried out at basic pH, combined with atmospheric pressure electrospray ionisation mass spectrometric detection in positive mode. The method had a limit of quantitation of at least 0.05 mg/L for all analytes in fluids and at least 0.05 mg/kg in solid tissues. This method was validated for all tissues analysed and was sufficiently accurate (within 80-120 % of target concentrations) and precise (intra-assay and inter-assay CV's ranging from 2.1 to 11 % and 3.2 to 13 %, respectively) for the determination of target drugs present in all postmortem cases included in this dissertation. Recoveries ranged 78-104 % for all drugs. The method was robust and provided the specificity and reproducibility required to obtain consistent results throughout the remainder of the presented experiments.

9.3. Drug of abuse assay development

Because of the frequency of substance abuse in people diagnosed with psychosis and/or depression, further method development was carried out to adapt the psychiatric drug analysis method to the determination of selected drugs of abuse. This was a more complex problem, since in contrast to psychiatric drugs, drugs of abuse comprise physicochemically diverse classes of compounds: amphetamines, benzodiazepines, cannabinoids, cocaine, and opiates. The ability to

detect these drugs in one analytical procedure was important since many of these cases also involved psychiatric drugs.

For sample preparation, a solid-phase extraction method described by Bogusz et al (Bogusz et al, 2000) was adapted for use with positive-mode electrospray LC-MS carried out at basic pH. Chromatographic conditions were only slightly modified from those used in the psychiatric drug analysis method, using a mobile phase with approximately 20% less methanol and a pH of 9.0 instead of 10.0. Results obtained with this method compared favourably to those obtained with techniques conventionally employed at the Institute's toxicology laboratory.   The method was useful for determination of a range of commonly abused drugs, although neither THC nor carboxy-THC were detected. This method provided adequate sensitivity, with a limit of detection of 0.05 mg/L for all drugs in blood. Recoveries obtained using this method expectedly spanned a wider range than those obtained for the psychiatric drugs (31-120 % for drugs of abuse compared to 78-104 % for psychiatric drugs). Adequate validation data with regard to accuracy (within 90-120 % of target) and precision (intra-assay and inter-assay CV's of 3-14 % and 14-19 %, respectively) were achieved.

The use of this method has been extended to the detection of over 40 basic drugs. When used to analyse 11 blood and urine specimens from coronial cases known to be positive for different drugs of abuse, results obtained using this method generally compared favourably to those obtained using conventional methods. If better detection sensitivity or selectivity than what was demonstrated in this method were desired, ion trap or tandem mass spectrometry could be employed, which would allow for MS/MS capability.

9.4. Postmortem tissue distribution of psychiatric drugs

Because little comprehensive data has been published on the tissue distribution of the target

psychiatric drugs, the next phase involved an investigation into this subject. The distribution

patterns of the target drugs and their major active metabolites were determined in a variety of

specimens obtained from individuals who had been taking such drugs. Target drug-positive cases

were included whether the drugs appeared to play a direct role in causing death or not. The

correlation of blood concentrations of these compounds to those measured in each tissue was also

examined.

Sertraline (n=21) and venlafaxine (n=20) were the two most commonly detected drugs in the

studied cases. The least common target drug was fluvoxamine (n=2).

Parent drugs were most often detected at highest concentrations in liver and bile, while the

highest metabolite concentrations were usually found in liver, frontal cortex, and bile,

respectively. Concentrations in blood, urine, and vitreous humour were the lowest. These results

are in accordance with what limited data on individual psychiatric drugs has previously been

published. Importantly, the investigation of the tissue distribution of multiple drugs showed

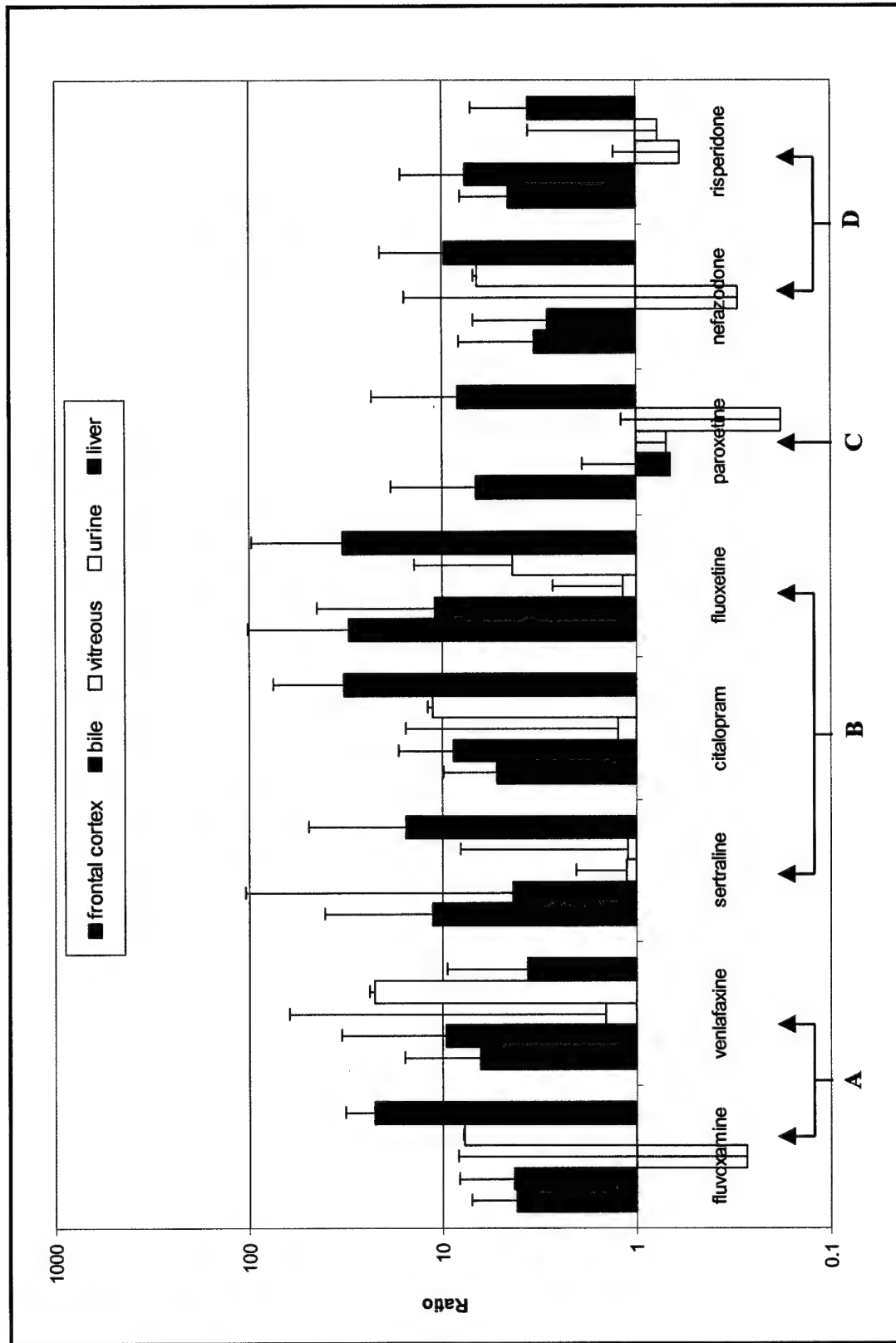different patterns which were summarised in Figure 9.1.

Figure 9.1.   Concentration ratios of target psychiatric drugs in tissues compared with femoral blood (means ±SD's).

In this study, four distinct patterns of distribution were noted, with drugs of similar molecular weights exhibiting the same pattern. Pattern A (urine > frontal cortex/bile > femoral blood > vitreous humour) was followed by fluvoxamine and venlafaxine. Pattern B (liver > frontal cortex/bile > urine/femoral blood > vitreous humour) was exhibited by sertraline, citalopram and fluoxetine. Paroxetine was the only drug to follow pattern C (liver > femoral blood > frontal cortex > bile > vitreous humour > urine), while pattern D (bile >frontal cortex/liver > urine/ femoral blood/vitreous humour) was seen for both nefazodone and risperidone.

On average, pattern A drugs were detected at approximately seven times higher concentrations in liver and four times higher in bile than in blood. By contrast, mean vitreous humour concentrations were 64% of those in blood. Drugs exhibiting pattern B were found at even higher concentrations in liver (13 times as high) and bile (5.5 times as high), although mean relative concentrations in vitreous humour were similar to those detected for pattern A drugs (67 % of those detected in blood). Paroxetine (pattern C) was found at lower concentrations compared to pattern A drugs in each tissue (140 %, 34 %, and 20 % for liver, bile, and vitreous humour, respectively). The mean liver concentrations for pattern D drugs were approximately eight times higher than those in blood, whereas mean bile concentrations were slightly over thirteen times higher. Mean vitreous humour concentrations approximated those found for paroxetine (29 %).

The data obtained for multiple psychiatric drugs enabled the investigation of the extent of correlation between the observed patterns and various pharmacokinetic parameters. Drugs with higher molecular weight were more likely to be found in bile. Target drug molecular weights

exhibited a statistically significant negative correlation with V_d, which may partially explain the differential distribution patterns observed. Target drugs with high molecular weights often had lower volumes of distribution and were only slightly correlated with bile: and liver:blood concentration ratios. With the exception of venlafaxine, tissues in which parent drug concentrations were significantly correlated with those in blood exhibited much lower correlations for metabolites. Such correlations were seldom statistically significant.

Blood concentrations of psychiatric drugs showed the highest correlations with those measured in vitreous humour and bile. The tissues that exhibited the lowest correlation with blood were liver and frontal cortex. Interestingly, frontal cortex:blood concentration ratios were significantly correlated with those of both liver:blood and bile:blood. Since psychiatric drug concentrations in these tissues were often much higher than those in blood, these tissues may be useful in determining past use.

9.5. Brain distribution of selected antipsychotic drugs

During the investigation of postmortem distribution of psychiatric drugs, it was noted that little had been reported on antipsychotic drug distribution in schizophrenic brains (Aravagiri et al, 1995; Merrick et al, 2001; Svendsen et al, 1988b; van Beijsterveldt et al, 1994). Further, technical differences between the few published studies make comparison of results difficult. Therefore, a study was undertaken to attempt to address whether selected antipsychotic drugs partition preferentially to particular brain regions in schizophrenics and whether any trends could be observed in the brain distribution patterns of antipsychotics as a class of drugs. Additionally,

the extent of correlation of blood concentrations with those determined in different brain regions was examined.

Brain regions selected for their known involvement in schizophrenia were collected at autopsy from 22 subjects who were confirmed to have schizophrenia in life. Antipsychotic drug concentrations were determined in these brain regions and compared for inter-subject variability.

From this investigation, it became obvious that phenothiazine antipsychotics distribute differentially in the schizophrenic CNS. If regional drug concentrations were normalised for those observed in cerebellum, three distinct patterns of distribution were observed, corresponding to different structural features of each type of phenothiazine. Group 1 (possessing an aliphatic side chain) and group 2 (possessing a piperidine ring in the side chain), were each associated with high affinity for dopamine receptors and were detected at highest concentrations in regions possessing high concentrations of such receptors (caudate-putamen and grey matter of the frontal cortex) (Figure 9.2). However, group 3 phenothiazines (with a piperazine ring in the side chain and associated with relatively lower dopaminergic activity than other neuroleptics) were found at highest concentration in occipital cortex, a region with a relatively low concentration of dopamine receptors.
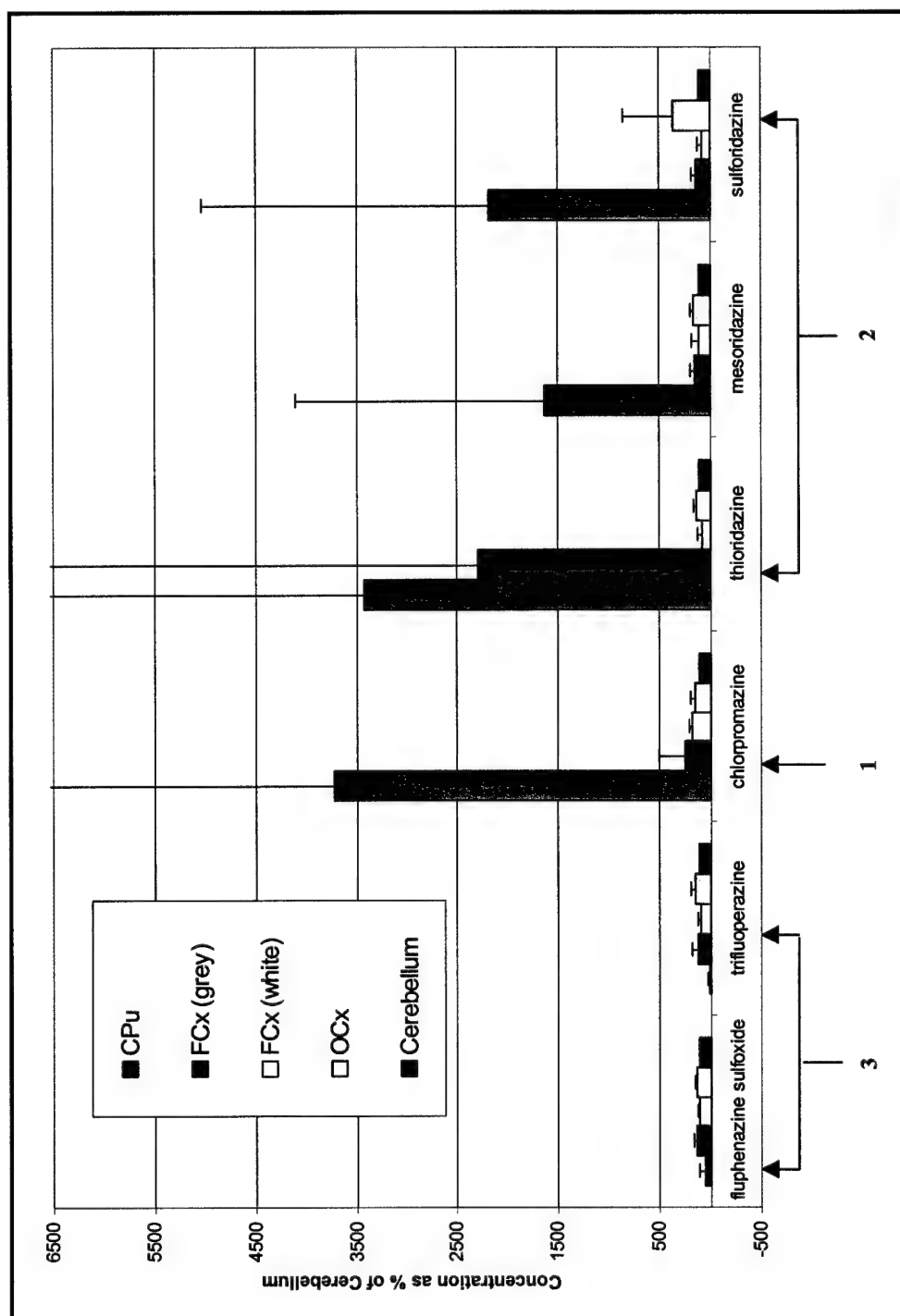
Figure 9.2. Mean regional brain distribution of selected antipsychotics relative to cerebellum concentrations and grouped according to structural features. Group 1: aliphatic side chain, Group 2: piperidine ring in side chain, Group 3: piperazine ring in side chain.

The regional brain distribution of thioridazine and its metabolites was dependant on overall concentrations in brain. In cases with moderate overall brain tissue concentrations, those detected in caudate-putamen were higher, whereas in a case with considerably higher overall concentrations, the concentration detected in occipital cortex was higher. This may suggest thioridazine initially distributes to regions of highest dopamine receptor concentration until active sites are saturated. Given this finding, it is notable that the mean absolute drug concentrations in caudate-putamen exhibited a highly significant correlation with lipophilicity, and that when corrected for cerebellum concentrations, those in occipital cortex were significantly correlated with volumes of distribution. Such correlations highlight the possibility of differential mechanisms for drug partitioning into the various regions of the brain, and provide the ability to better understand their distribution into these two regions.

Normalised regional metabolite concentrations followed the same distribution pattern as parent drug in the cases containing the three highest overall thioridazine concentrations. In the fourth case, in which lower concentrations of all three compounds were detected, patterns of distribution differed. This supports Svendsen et al's findings (Svendsen et al, 1988b) in a patient who was withdrawn from thioridazine who began to exhibit some regional partitioning of parent drug and metabolites in brain, and suggests differential rates of clearance between brain regions for drugs.

It is interesting to note that in almost half of the brains analysed in this study, residual antipsychotic drugs were not detected using the limit of detection of 10 ng/g. This may be reflective of schizophrenic subjects withdrawing from their medication prior to death, and has important implications for interpreting the role of antipsychotic drugs in deaths. Low or

undetectable concentrations of antipsychotic drugs in blood relative to their therapeutic windows corresponded to low concentrations in selected regions of the CNS, such that relative concentrations measured in blood are somewhat reflective of those in various brain regions. There was not enough data in this study to test for the correlation of individual drug concentrations in blood to those in brain tissue. However, if data for chlorpromazine, thioridazine, and trifluoperazine were combined, significant positive correlations between concentrations in blood and those in white matter of the frontal cortex, occipital cortex, and cerebellum were observed. It will be interesting to see if future studies support these observations.

9.6. Postmortem redistribution of psychiatric drugs

A study was conducted to assess the degree to which psychiatric drugs and their metabolites are subject to the process of postmortem redistribution. Previous studies showed the highest mean drug concentration ratios in heart:femoral blood in fluoxetine and fluvoxamine (3.1 for each drug), while those of paroxetine were the lowest (heart:femoral concentration ratio = 1.2) (Fu et al, 2000; Hilberg et al, 1992b; Levine et al, 1994; Levine et al, 1996; Logan et al, 1994; Parsons et al, 1996; Vermeulen, 1998). However, these studies do not provide sufficient data to draw conclusions regarding redistribution of the target psychiatric drugs as a group. This lack of information makes it difficult to interpret drug concentrations measured in postmortem cases, particularly when the postmortem interval (PMI) is particularly long.

Redistribution was investigated by comparing postmortem blood concentrations from two different collection sites at autopsy (blood from the heart and femoral regions). Femoral blood concentrations were also compared to length of PMI. Amounts of drug residue in stomach contents were determined and compared to corresponding concentrations in blood and liver to investigate the possibility of drug diffusion from the gastrointestinal tract (GIT) into central blood or liver.

There were not enough specimens in which blood from both sampling sites was collected to assess the psychiatric drugs individually for statistical significance of concentration differences. However, heart blood concentrations were significantly higher (34 %, on average) than those measured in femoral blood when results from all drugs were included together.

It was of interest that the heart blood specimens in three cases were visibly lighter in colour and therefore thinner than their corresponding femoral blood specimens. These three cases accounted for the highest heart:femoral blood concentration ratios. The high lipophilicity of the target psychiatric drugs enables them to distribute extensively into red blood cells, which would not explain the significantly higher heart blood concentrations in these cases. Furthermore, Amitai et al have published evidence that suggests plasma concentrations of SSRIs are more similar to those of whole blood than the TCAs, in which red blood cells are thought to dilute detected plasma concentrations (Amitai et al, 1993).

Although not significantly different to those determined for other target drugs, the highest heart:femoral ratios were observed with fluoxetine, paroxetine, and risperidone. No particular difference in mean heart:femoral ratios was observed for citalopram, sertraline, or venlafaxine.

The findings from this study accord with previously published studies although other studies have reported higher heart:femoral ratios for citalopram and venlafaxine (Figure 9.3) (Fu et al, 2000; Levine et al, 1994; Logan et al, 1994; Parsons et al, 1996; Vermeulen, 1998). The differences between these studies may reflect the variability that exists between cases.

Small amounts of drug residue were detected in the stomach contents from the studied cases. Therefore, it is not possible to determine if diffusion from the GIT contaminated centrally collected blood or liver. A slight difference between heart:femoral ratios was observed between cases where drug and metabolite concentrations in liver were >1.0 mg/kg and those in which liver drug concentrations were <1.0 mg/kg. This suggests drug diffusion from solid tissues may occur, leading to some degree of redistribution.

No significant correlation was observed between heart:femoral concentration ratios and volume of distribution (V_D), degree of protein binding (F_b), or lipophilicity (logP). This may explain why no significant difference in heart and femoral blood concentrations was observed for sertraline even though its values for each of these parameters are high. Blood being taken from a femoral site without first ligating the vessel could also account for this similarity in drug concentrations between the two types of blood. In the presented cases, the femoral vessel was not ligated prior to sampling, although care was taken to minimise the possibility of contamination of blood from central tissues.
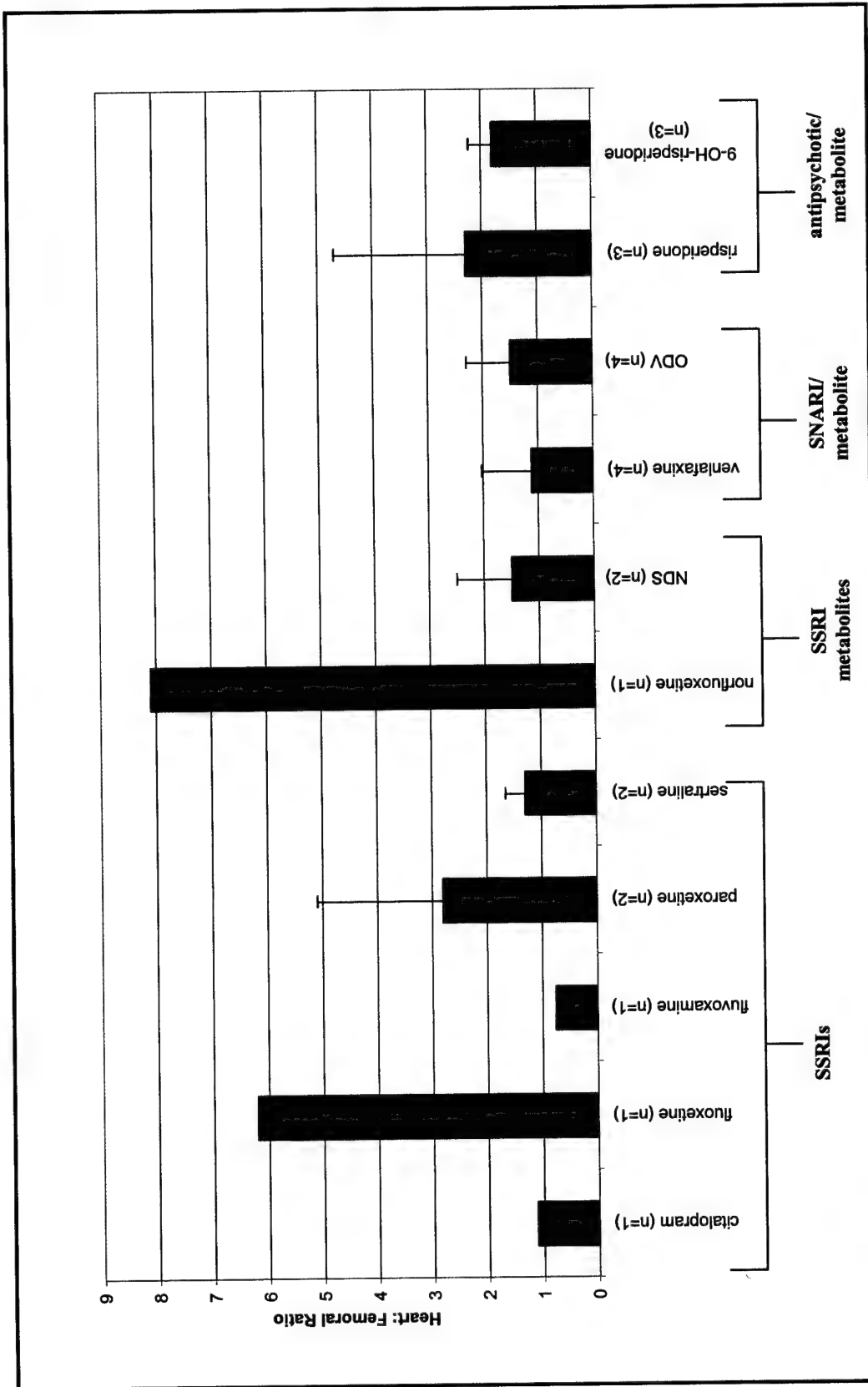
Figure 9.3. Mean heart: femoral concentration ratios obtained in this dissertation.

Based on the results from this study, it seems that the redistribution observed for the target psychiatric drugs is largely driven by drug diffusion from tissues and solid organs containing high concentrations to areas of lower concentration. It should be noted that even femoral blood concentrations could be elevated postmortem, particularly when the PMI is long. Drug concentrations in the presented cases with longer PMI's were slightly higher than those in the remaining cases. However, this difference was not significant. Further studies should be undertaken in which femoral blood samples are taken at admission to the mortuary and compared to those obtained at autopsy in order to more fully assess this phenomenon. This was not possible at the Institute due to the need to obtain senior next-of-kin permission prior to obtaining specimens.

It is widely accepted that femoral blood is the specimen of choice for toxicological analysis (Forrest, 1993; Hearn et al, 1991; Jones and Pounder, 1987; Patterson, 1993; Pounder and Jones, 1990; Prouty and Anderson, 1990). Because redistribution seems to occur with the target drugs, femoral blood should be taken in preference to blood from other regions to minimize the possibility of contamination from solid tissues and other body fluids.

9.7. Trends in cause of death

Throughout this dissertation it was recognised that the target psychiatric drugs might be associated with varying risks of toxicity. The relative potency of typical antipsychotics has been characterised by Foster (Foster, 1989) and enjoys broad general acceptance by practitioners and clinicians alike. However, no clear association between dose and clinical effect has been

demonstrated for the target antidepressants. Because of this, it is difficult to appreciate the magnitude of risk associated with these drugs. Therefore, the final portion of my dissertation involved an investigation into trends in circumstances of death where the target psychiatric drugs were detected. The ultimate objective of this phase of my research was to highlight possible risk factors associated with these drugs.

Case information obtained from police reports, toxicological and pathological findings, and coronial inquests was examined and circumstances of death collated in 76 death investigation cases discussed throughout this dissertation. Cases were categorised according to cause of death Deaths attributed to heroin toxicity were excluded from the analysis. The relative contribution to cause of death for each target drug was estimated by comparing detected concentrations in blood to those reported in postmortem case reports of those appearing to have arisen from therapeutic use. In cases where such data was unavailable, detected concentrations were compared to the maximum plasma concentrations reported in clinical trials resulting from therapeutic use. Drug concentrations in each death were categorised as either

1) therapeutic and not likely to have caused death,

2) therapeutic but drug's serotonergic activity may have enhanced effects of other drugs,

3) supratherapeutic but not likely to have contributed to death,

4) supratherapeutic and likely to have contributed to death, or

5) the drug's contribution to death was unclear.

This was adopted from that agreed to by a meeting of Australasian senior toxicologists during the development of a National Coroners' database.

Using this contribution scoring system, there were 21 instances of a target drug being scored as a major contributor and 17 instances in which a target drug was considered to be a minor contributor due to their potential serotonergic activity. Venlafaxine and paroxetine were most commonly implicated as major contributors (contribution scores = 4), and the most common minor contributors (receiving contribution scores = 2) were fluoxetine, risperidone, and sertraline.

Pathological findings at autopsy were of limited use in interpreting the role of psychiatric drugs in causing death. This was the case since most deaths involved multiple drugs, and autopsy findings were usually reflective of natural disease states observed in people with histories of drug abuse or psychiatric conditions. In cases where the serotonergic activity of the target drugs may have played a direct role, three types of conditions were noted:

a) Evidence of extensive coronary artery disease, particularly in cases where extensive ischaemic damage was observed, which may be an indication that cardiac complications due to the presence of one or more of the target drugs occurred.

b) Evidence of haemorrhaging, epistaxis, haematemesis, or other bleeding disorders, or of splenomegaly, which may indicate target drug-induced changes in platelet serotonin concentrations, affecting clotting mechanisms.

c) Evidence of serotonin syndrome such as organ failure and rhabdomyolysis, or
pulmonary oedema.

In drug-related deaths, cases were grouped into the following categories:

d) deaths in which a target drug was detected in combination with an MAOI (n=5),

e) deaths involving a combination of more than one target drug (n=7), and

f) deaths in which a target drug was detected in combination with other types of drugs
(n=5) (see Figure 9.4).

This is especially troubling since such combinations are specifically warned against in the prescribing information for these drugs (Caswell and et al, 2001; The United States Pharmacopoeial Convention, 1995). There were more than twice as many females as males represented in the drug-related deaths. It is notable that subjects in 49 of the 72 cases were noted to have physical ailments that may have been worsened by the presence of serotonin-active drugs. This includes subjects with cardiovascular disease (n=16), some form of hepatic impairment (n=25), and different types of haemorrhaging (n=8).
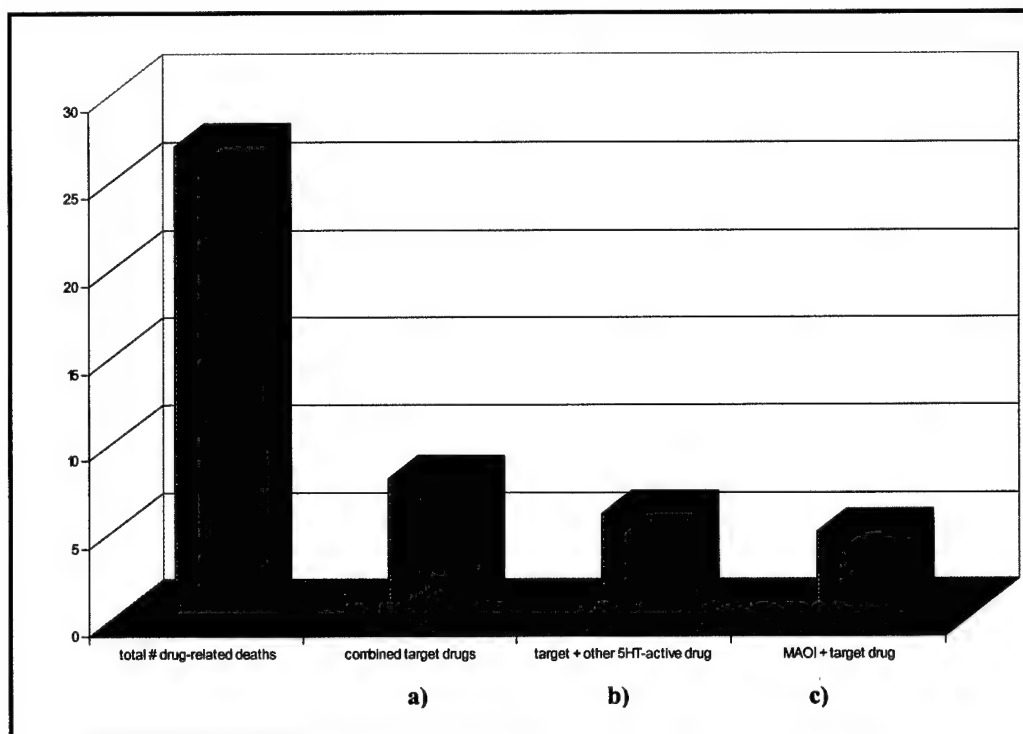
Figure 9.4. Relative number of drug-related deaths in which possible adverse drug combinations were detected.

Cardiac vasoconstriction due to the presence of one or more serotonergic drugs may have contributed to death in six natural deaths and four drug-related deaths, including one in which an antiepileptic (gabapentin) was detected in combination with a target drug. In these cases, the extensive ischaemic disease of the coronary arteries noted at autopsy could lead to direct unopposed vasoconstriction caused by serotonin and modulated by 5-HT$_{1A}$ receptors (Golino et al, 1991).

Case circumstances in another five drug-related deaths and three non-drug accidents suggest the subjects may have developed bleeding disorders prior to death which were exacerbated by one or more target drugs. Such bleeding complications arise due to the presence of drugs that inhibit

serotonin reuptake, resulting in decreased concentrations of circulating serotonin. Target drug-related bleeding disorders also appear to have been implicated in four of the non-drug accidents, as the subjects suffered haemorrhaging or blood loss associated with complications of recent operations or injuries (Aranth and Lindberg, 1992; Fischer et al, 1995; Goldberg, 1998; Shapiro, 1996; Yaryura-Tobias et al, 1991).

There were 13 drug-related deaths in which the subject may have developed serotonin syndrome prior to death. In five of these cases an MAOI (such as moclobemide) was considered to play a major role in causing death in combination with one or more target drugs, due to their known propensity to cause serotonin syndrome.   In the remaining eight cases, target drugs were detected in combination or with other drugs. Interestingly, the subject in one such case was found to have an elevated serum valproic acid concentration, which was sufficient to have caused death alone. In combination with other serotonergic drugs, it is also possible the subject developed serotonin syndrome prior to death.

CYP450 isoenzyme metabolic inhibition does not appear to have been clinically relevant in any cases. There were four cases in which drugs present had the potential to inhibit CYP 2D6. However, the contribution of enzyme inhibition to toxicity is difficult to ascertain since such inhibition would result in the development of serotonin syndrome due to the combination of MAOIs or TCAs with target drugs.

These results highlight that multiple target psychiatric drugs were present in most subjects, which raises the possibility of either inadequate control leading to polypharmacy or deliberate misuse of these drugs.

9.8. Summary and conclusion

In summary, my research showed

Assay development

- Psychiatric drugs and their active metabolites can be separated and detected using an isocratic reversed-phase LC-MS method at basic pH in a variety of postmortem specimens using a liquid-liquid extraction method.
- Drugs of abuse can also be detected using solid-phase extraction and an instrumental method only slightly modified from that employed for psychiatric drug determination.

Postmortem tissue distribution of psychiatric drugs

- The highest concentrations of psychiatric drugs were generally found in liver and bile, while the lowest concentrations were found in blood, urine, and vitreous humour.
- Four distinct patterns of distribution were noted, with drugs of similar molecular weights exhibiting similar patterns: A) urine > frontal cortex/bile > femoral blood > vitreous humour; B) liver > frontal cortex/bile > urine/femoral blood; C) liver > femoral blood >

frontal cortex > bile > vitreous humour > urine; and D) bile > frontal cortex/liver > urine/femoral blood/vitreous humour.

- In general, higher molecular weight drugs were found at relatively high concentrations in bile and lower concentrations in liver in comparison to other drugs. Molecular weights showed a significant negative correlation with volume of distribution.

- Blood concentrations of psychiatric drugs were most highly correlated with those measured in vitreous humour and bile. The lowest blood:tissue correlations were observed with liver and frontal cortex.

- A positive, significant correlation between vitreous:blood concentration ratios and those in urine was observed, possibly due to the high water content of both tissues.

- Frontal cortex:blood concentration ratios exhibited significant positive correlations with those of liver and bile.

- The metabolites of target drugs generally showed little correlation with parent drug concentrations and between tissues.

Brain distribution of antipsychotic drugs in schizophrenics

- Three distinct patterns of regional distribution were noted. Neuroleptics with higher dopaminergic activity were found at higher concentrations in regions with more dopamine (DA) receptors. Those with lower dopaminergic activity were found in regions possessing fewer DA receptors.

- The regional brain distribution of thioridazine and its metabolites depended on overall concentrations in brain. When overall concentrations were high, metabolite partitioning

followed that of the parent drug. When overall concentrations were low, distribution differed for each compound, reflecting differential clearance rates between brain regions.

- Positive significant correlations were observed between mean drug concentrations in caudate-putamen and lipophilicity, as well as between concentrations in occipital cortex (corrected for those in cerebellum) and volume of distribution. This suggests the extent of antipsychotic drug partitioning into these regions can (in part) be predicted using these parameters.

- Low or undetectable concentrations of antipsychotic drugs in blood relative to their therapeutic range corresponded to low concentrations in the CNS. Positive, significant correlations were observed between concentrations in blood and those in grey matter of the frontal cortex, occipital cortex, and cerebellum, suggesting concentrations of antipsychotics in these regions can loosely be inferred from those detected in blood, and vice-versa.

Postmortem redistribution of psychiatric drugs

- Target drug concentrations were on average between 2-3 times higher in heart blood than in femoral blood, indicating these compounds undergo some postmortem redistribution. Heart:femoral ratios for all target drugs ranged from 0.50-6.2.

- There was a slight increase in drug concentrations in cases with longer PMI's which was not significant. It was not possible to determine the extent to which drug concentrations changed during the postmortem interval (PMI), which averaged 62 hours.

- No significant correlations between heart:femoral ratios and volume of distribution, degree of protein binding, or lipophilicity were observed.

- Heart:femoral ratios in cases where target drug concentrations in liver were >1.0 mg/kg were significantly different to those in cases where liver drug concentrations were <1.0 mg/kg. This suggests drug diffusion from solid tissues occurs and may be responsible for at least some redistribution of the target psychiatric drugs.

Trends in cause of death

- Subjects in drug-related deaths involving psychiatric drugs, whether accidents or suicides, were mostly females. Approximately 71% of the presented cases involved females.

- Pathological findings indicating target drugs may have played a role in death include evidence of cardiac vasoconstriction, bleeding disorders (including splenomegaly), or serotonin syndrome (evidenced by organ failure or rhabdomyolysis).

- Using the contribution scoring system, venlafaxine and paroxetine were the most commonly implicated of the target drugs as being major contributors to death. Fluoxetine, risperidone, and sertraline were regarded as minor contributors to toxicity in drug-related deaths.

- Cardiac complications potentially caused by the presence of one or more target drugs may have contributed to death in a further four drug-related deaths and six natural deaths.

- Bleeding disorders due to changes in platelet serotonin concentrations may have played a role in five drug-related deaths and three non-drug accidents.

- Serotonin syndrome could have occurred in 13 out of 71 selected cases. In these cases, target drugs were detected in combination with each other or with drugs known to be capable of exacerbating serotonin syndrome, such as MAOIs, TCA's, or antiepileptics.

- Although drugs capable of causing inhibition CYP450 isoenzymes were detected in four cases, it is unlikely any such inhibition was clinically relevant. Since enzyme inhibition in these cases would possibly potentiate serotonin syndrome, which may have occurred regardless of such inhibition, it is difficult to determine the extent to which it may have contributed to toxicity.

- Almost all subjects were using more than one serotonin-active drug, indicating the possibility of either inadequate control or deliberate misuse of these drugs. Accidental toxicities comprised 16 out of 27 drug-related deaths, suggesting target drug-related deaths are more often than not unintentional.

- In none of 85 cases was a single target psychiatric drug the sole cause of death. However, in 38 cases, one or more target drugs were considered to have played at least a minor role.

In conclusion, it is hoped that these studies have shown the usefulness of new technologies such as LC-MS in detecting a number of monoamine-active drugs that were not easily detected simultaneously using conventional analytical methods. It is also hoped that these findings will broaden our understanding of the toxicology of psychiatric drugs.

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Foster's paper on neuroleptic equivalence

Neuroleptic equivalence

By PAUL FOSTER, BSc, MRPharmS, CHSM

The stated relative potencies of antipsychotic drugs appear to vary significantly between different information sources. This may lead to confusion when deciding upon the most appropriate dose, either when initiating antipsychotic therapy or when changing from one antipsychotic drug to another. This paper compares the published data on the relative potencies of the antipsychotic drugs with the opinions of psychiatrists in Leicestershire health authority and with the recommendations of the pharmaceutical industry.

THE antipsychotic drugs are primarily used for the treatment of psychosis and the prevention of psychotic relapse in schizophrenic patients. The drugs vary in efficacy and in the frequency and degree of their side effects (for example, sedation and anticholinergic and extrapyamidal effects).

Comparison of therapeutic efficacy is usually made by reference to a standard dose of chlorpromazine.[1-29] Manufacturers often recommend their products to be equivalent in antipsychotic effect to standard doses of chlorpromazine and two companies have issued specific dosage calculators.

Survey

A questionnaire (Figure 1) was sent to 26 consultants and senior registrars working in

▶ *Mr Foster is staff pharmacist, Towers hospital, Leicester*

psychiatry in Leicestershire health authority. It asked what, in their opinion, were the eqivalent antipsychotic doses of the commonly used neuroleptic drugs both in oral and in depot form compared to an oral dose of chlorpromazine.

Eighteen questionnaires were returned and the results tabulated, (Tables 1a and 1b). Literature values[1-29] are shown in Table 2 and values obtained from the manufacturers are shown in Tables 3 to 6. Published literature on the equivalence of five of the agents included in the clinical questionnaire was not available in sufficient quantity to make it reliable. Fewer agents are, therefore, shown in Tables 3 to 7.

A summary of the results is presented in Tables 7 and 8.

Two dosage calculators in the form of discs are currently being distributed by Lundbeck and by Squibb. The equivalent doses each recommends are shown in Tables 5 and 6.

Discussion

Oral Drugs There appears to be a general agreement on the relative potencies of the oral neuroleptics quoted (i) in the literature, (ii) by the pharmaceutical industry and (iii) in clinical practice. The notable exception to this is haloperidol. Janssen advocates 2mg haloperidol to be equivalent in antipsychotic effect to 100mg chlorpromazine and this is supported by most of the literature. However, a sample of 18 psychiatrists thought 10mg haloperidol to be equivalent to 100mg chlorpromazine.

There could be three reasons for this discrepancy. First, confusion between antipsychotic activity and the sedative properties of a neuroleptic would tend to show haloperidol to be less potent in comparison to chlorpromazine. It is important to emphasise that sedation does not correspond to antipsychotic activity.

Secondly, haloperidol is used in high

Table 1 (a): Results of the clinical practice questionnaire on oral drug equivalents

Drug	Median dose (mg) equivalent to 100mg chlorpromazine	Range (mg)
Droperidol	10	10-20
Flupenthixol	4.5	3-6
Fluphenazine	4	2-10
Haloperidol	10	2-20
Pericyazine	10	10-25
Perphenazine	8	4-10
Pimozide	4	4-10
Promazine	100	50-150
Sulpiride	300	100-800
Thioridazine	100	50-100
Trifluoperazine	5	5-10

Table 2: Dose equivalents found in literature search

Drug	Median dose (mg) equivalent to 100mg chlorpromazine	Range (mg)
Fluphenazine [1,7-10,18-20,25,27]	2	2-5
Haloperidol [5,7-10,18,20,27]	2	1.5-5
Perphenazine [1,8-10,18,25,27]	10	5-12
Promazine [1,7-10,19,27]	200	100-250
Thioridazine [5,7-10,18,19,25]	100	50-120
Trifluoperazine [1,5,7-10,18,19,25,27]	5	3.5-7.5

Table 1 (b): Results of the clinical practice questionnaire on depot drug equivalents

Drug	Median fortnightly dose (mg) equivalent to a daily oral dose of 300mg chlorpromazine	Range (mg)
Clopenthixol	200	100-600
Flupenthixol	40	20-60
Fluphenazine	25	25-50
*Fluspirilene	4	4-8
Haloperidol	100	20-200
Pipothiazine	50	50-100

* In practice fluspirilene would be administered on a weekly basis

Table 3: Oral doses recommended as equivalent in antipsychotic activity to 100mg chlorpromazine by pharmaceutical companies

Oral drug	Dose equivalent to chlorpromazine 100mg
Haloperidol (Janssen)	2mg
Perphenazine (A&H)	10mg
Promazine (Wyeth)	100-200mg
Sulpiride (Squibb)	200mg
Thioridazine (Sandoz)	100mg
Trifluoperazine (SK&F)	4mg

Table 4: Depot doses recommended by Janssen and Lundbeck as equivalent in antipsychotic effect to 100mg of oral chlorpromazine

Depot drug	Dose equivalent to oral chlorpromazine daily dose 100mg		
	Janssen	Lundbeck	
Clopenthixol	80mg	200mg	
Flupenthixol	16mg	40mg	Every
Fluphenazine	10mg	25mg	two
Haloperidol	20mg	—	weeks
Pipothiazine	20mg	—	

Table 5: Equivalent antipsychotic doses recommended by the Lundbeck disc

Drug	Dose	
Chlorpromazine	100mg	
Clopenthixol	200mg	IM every two weeks
Fluphenazine	25mg	
Flupenthixol	40mg	
Haloperidol	5mg	
Thioridazine	100mg	

Table 6: Equivalent oral antipsychotic doses, recommended by the Squibb disc

Drug	Dose
Chlorpromazine	600mg
Haloperidol	30mg
*Thioridazine	600mg
Trifluoperazine	15mg
Sulpiride	1200mg

* This disc also recommends 800mg thioridazine as equivalent to 1200mg chlorpromazine

Equivalence of Antipsychotic Drugs

Name Dr...

Hospital...

Please state what, in your opinion, is the equivalent antipsychotic dose of the following oral agents compared to an oral dose of 100mg chlorpromazine.

Chlorpromazine	100mg
Droperidol	... mg
Flupenthixol tablets	... mg
Fluphenazine tablets	... mg
Haloperidol	... mg
Pericyazine	... mg
Perphenazine	... mg
Pimozide	... mg
Promazine	... mg
Sulpiride	... mg
Thioridazine	... mg
Trifluoperazine	... mg

Please state what in your opinion is the depot dose equivalent in antipsychotic effect to a daily oral dose of 300mg chlorpromazine.

Flupenthixol	... mg every two weeks
Fluphenazine decanoate	... mg every two weeks
Fluspirilene	... mg weekly
Haloperidol decanoate	... mg every two weeks
Pipothiazine	... mg every two weeks
Clopenthixol	... mg every two weeks

Figure 1: The neuroleptic equivalence questionnaire

Table 7: Summary of results for oral drug equivalents

Drug	Median dose (mg) equivalent to 100mg chlorpromazine		
	Literature search	Pharmaceutical industry	Clinical practice
Fluphenazine	2	—	4
Haloperidol	2	2	10
Perphenazine	10	10	8
Promazine	200	150	100
Thioridazine	100	100	100
Trifluoperazine	5	4	5

Table 8: Summary of results for depot drug equivalents

Drug	Fortnightly depot dose (mg) equivalent to a daily oral dose of 100mg chlorpromazine		
	Lundbeck	Janssen	Clinical practice (median)
Clopenthixol	200	80	67
Flupenthixol	40	16	13
Fluphenazine	25	10	8
Haloperidol	—	20	33
Pipothiazine	—	20	17

doses in acute mania, where it is not used as a typical antipsychotic. This could cause clinicians to view it as a less potent antipsychotic than it really is.

Thirdly, the relationship between haloperidol dose and the equivalent chlorpromazine dose is unlikely to be linear. Using positron emission tomography, various authors have shown the majority of brain dopamine receptors to be occupied by relatively small doses of haloperidol.[14,28] Farde showed 84 per cent of D_2 receptors to be occupied following an 8mg daily dose of haloperidol. It would therefore be reasonable to assume that the relative antipsychotic potency of haloperidol will significantly decrease as the dosage increases.

Depot drugs There is a large discrepancy between the recommendations of Lundbeck and Janssen when extrapolated to an oral chlorpromazine equivalent. However, the companies agree on the relative potencies of clopenthixol to flupenthixol to fluphenazine. There is also a large discrepancy between the recommendations of Lundbeck and what is found in clinical practice. However, there is general agreement between the recommendations of Janssen and the views of the clinicians, apart from on haloperidol decanoate. Janssen believes this to be significantly more potent than do the clinicians.

Dosage calculators The Lundbeck disc recommendations for oral antipsychotic drugs concurs with the literature. As discussed above, the Lundbeck recommendations for depot antipsychotics differ significantly from what is found in clinical practice in Leicester, with Lundbeck believing its depots to be less potent than do the clinicians. The Squibb disc is more controversial. It suggests trifluoperazine to be twice as potent as haloperidol, which is not substantiated in the literature. It also makes thioridazine in high dosage appear substantially more potent than chlorpromazine. Again, this is not supported by the literature.

The study indicates that the Squibb dosage calculator is acceptable for sulpiride but is misleading for other neuroleptic drugs and should be disregarded.

Conclusion

It would appear that the consensus is that the agents shown in Tables 9 and 10 have an equivalent antipsychotic effect.

As regards the clinical implications of the findings, it is important to emphasise that there is a great inter-patient variation when considering what dose of a neuroleptic in appropriate. However, when converting from one neuroleptic to another it is important that the dosage is calculated accurately

Table 9: Oral drug equivalents

Drug	Equivalent dose
Chlorpromazine	100mg
*Haloperidol	3mg
Perphenazine	8mg
Promazine	100mg
Thioridazine	100mg
Trifluoperazine	5mg

* This conversion may be appropriate only at doses below 20mg per day; at higher doses the relative potency of haloperidol may decrease

to avoid either psychotic relapse or overdosage. It is in the sphere of neuroleptic substitution that the findings of this study can be best put into clinical practice, especially when considering the potencies of depot preparations and haloperidol, where there was the greatest variation between clinicians' beliefs and the findings of controlled scientific studies.

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Table 10: Depot drug equivalents

Drug	Equivalent dose	
Chlorpromazine	100mg	daily (oral)
Clopenthixol	80mg	
Flupenthixol	16mg	
Fluphenazine	10mg	IM every two weeks
Haloperidol decanoate	30mg	
Pipothiazine	20mg	

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Plain terms explanation of research (given to senior next of kin)

EC 199   **Approval Date: 30ᵗʰ September 1999**

Researcher: Kabrena Goeringer (VIFM)

Toxicology of atypical antidepressants with serotonergic activity
(written by researcher)

This project aims to help future investigations into how different drugs may contribute to death by looking at the way antidepressant drugs are released into various organs in the body. Presently most doctors believe that antidepressant drugs (like Prozac) are very safe even if a person takes more than the prescribed dose, while taking other drugs. The findings from this research will help identify whether it is dangerous to prescribe and take different drug combinations.

The samples we need to test are blood, bile, urine and vitreous fluid (from the back of the eye) – about 10 mL of each of these, stomach contents, and small samples of liver and brain. These samples are normally taken during the autopsy for diagnosis of the cause of death. With the permission you have given, we will do these extra tests on the samples for this project. We will compare the results of the tests in about 100 cases to see if trends exist. After the tests are completed some of the samples will be stored at the Victorian Institute of Forensic Medicine, in case further tests need to be done to find out the cause of death. We will make sure that the rest of the samples are disposed of safely and respectfully.

Thank you for considering tissue donation for this project. By deciding to donate you will be contributing to research that aims to assist in preventing people dying unnecessarily from taking dangerous drug combinations.

Validation of calculations using the PALLAS Expert System

Prediction of Distribution Coefficient from Structure. 1. Estimation Method

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Received April 18, 1996, from the [†]CompuDrug Chemistry Ltd., 1362 Budapest 62, P.O.B. 405, Hungary, [‡]University of Athens, Department of Pharmacy, Division of Pharmaceutical Chemistry, Panepistimiopoli, Zografou, Athens, 157 71, Greece, and [§]Comgenex Inc., Bem rkp. 33/34, Budapest, H-1027, Hungary.   Accepted for publication April 29, 1997[®].   [x]Present address of corresponding author: Medical Sciences Building, Department of Pharmacology, University of Toronto, Toronto, M5S 1A8, Canada. Tel: (416) 978-2044. Fax: (416) 978-6395. e-mail: f.csizmadia@utoronto.ca.

Abstract ☐ A method has been developed for the estimation of the distribution coefficient (*D*), which considers the microspecies of a compound. *D* is calculated from the microscopic dissociation constants (microconstants), the partition coefficients of the microspecies, and the counterion concentration. A general equation for the calculation of *D* at a given pH is presented. The microconstants are calculated from the structure using Hammett and Taft equations. The partition coefficients of the ionic microspecies are predicted by empirical equations using the dissociation constants and the partition coefficient of the uncharged species, which is estimated from the structure by a Linear Free Energy Relationship method. The algorithm is implemented in a program module called PrologD.

Lipophilicity is a physicochemical property that has attracted considerable interest in medicinal chemistry and environmental sciences. Hydrophobic interactions with receptors, penetration across biological membranes during drug transport, as well as toxic aspects of drug action underline the important role of lipophilicity in drug research.[1-3] Additionally, soil sorption, aquatic toxicity, bioaccumulation, and biodegradation processes reveal the effect of lipophilicity in the environmental fate of chemicals.[4,5] The octanol–water partition coefficient, used in its logarithmic form (log *P*), is the most widely accepted measure of lipophilicity and refers to the partitioning of the same species of a substance between octanol and the aqueous phase.[6,7] However, substances which contain ionogenic functions may exist as a mixture of the dissociated and undissociated forms at different pH values. In such cases the apparent partition coefficient or distribution coefficient *D* (mostly used as log *D*), which refers to more complex partitioning equilibria,[1,8] is considered.

The considerable limitations in partitioning experiments have led to the development of calculative approaches, mostly restricted to the prediction of the partition coefficient of the uncharged species.[9-12] However, since biological and chemical systems are characterized by different pH values, distribution coefficients (log *D*) are frequently required.

In order to enable predictions of the distribution coefficient at any pH, a program module called PrologD[13-15] has been developed, which is a part of the Pallas program.[16] (Pallas integrates modules that predict physicochemical parameters from structure.) The method estimates log *D* from the two-dimensional structure of the microspecies of compounds.

Theoretical Section

Several microspecies of a compound may exist in a protic solvent. As an example, the microspeciation of leucinylphenylalanine (Leu-Phe), a dipeptide with one acidic and one basic group, is shown in Figure 1.
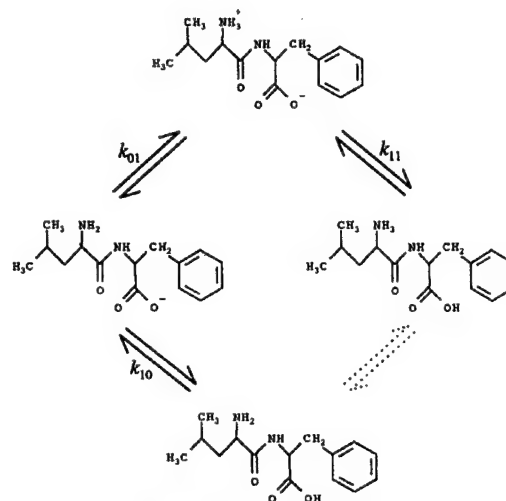
Figure 1—Protonation scheme of Leu-Phe.

Equations 1 and 2 describe the protonation and partitioning processes by macroconstants for mono- and diprotic compounds.

Monoprotic case

$$D = \frac{P_0}{1 + [H^+]K_0} + \frac{P_1}{1 + \dfrac{1}{[H^+]K_0}} \tag{1}$$

Diprotic case:

$$D = \frac{P_0}{1 + [H^+]K_0 + [H^+]^2 K_0 K_1} + \frac{P_1}{1 + \dfrac{1}{[H^+]K_0} + [H^+]K_1} +$$

$$\frac{P_2}{1 + \dfrac{1}{[H^+]^2 K_0 K_1} + \dfrac{1}{[H^+]K_1}} \tag{2}$$

where *D* is the distribution coefficient, the P_I and K_I values are partition and protonation macroconstants, respectively.

$$P_I = \frac{C_I^o}{C_I} \tag{3}$$

$$K_I = \frac{C_{I+1}}{[H^+]C_I} \tag{4}$$

Figure 2—Protonation scheme of a diprotic compound.

In eqs 3 and 4, I refers to the number of protonated groups in the species and C_I and C_I^o are the sums of concentrations of microspecies with the same net charge (characterized by I) in water and octanol, respectively. In pH ranges where the partitioning of one or two macrospecies is negligible, equations simpler than 1 or 2 can be used. The equation for the calculation of the distribution coefficient becomes much more complex if the number of protonation sites is more than two. The general equation is shown in the Appendix (see eq 3).

While protonation and partition macroconstants are good descriptors of the overall acidity, basicity, and lipophilicity of the molecule, the proton binding ability and lipophilicity of the various protonation states of the molecules are characterized by the protonation[17-20] and partition[21-24] microconstants. Moreover, macroconstants cannot be predicted directly because knowledge of the location of the charges is necessary for the prediction. The micro- and macroconstants are the same in the case of monoprotic compounds, but the number of microconstants increases exponentially with the number of the protonation sites. Figure 2 shows the generalized micro- (top) and mac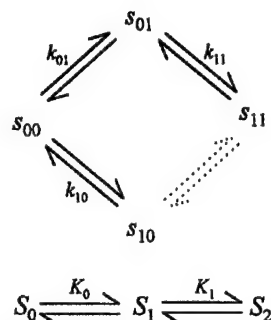ro- (bottom) protonation schemes of a diprotic compound using a binary indexing of species and constants which is detailed in the Appendix (the protonation constant represented by the dashed arrow is not needed for the calculations).

s_i and S_I refer to the ith microspecies and Ith macrospecies, respectively (small letters are used for the indices and constants of microspecies and capital letters for the macrospecies). For a diprotic compound, macroconstants can be calculated from microconstants by eqs 5–9 (for the general case, see eqs 34 and 35 in the Appendix):

$$K_0 = k_{01} + k_{10} \qquad (5)$$

$$K_1 = \frac{k_{01}k_{11}}{k_{01} + k_{10}} \qquad (6)$$

$$P_0 = p_{00} \qquad (7)$$

$$P_1 = \frac{p_{01}k_{01} + p_{10}k_{10}}{k_{01} + k_{10}} \qquad (8)$$

$$P_2 = p_{11} \qquad (9)$$

where p_i and k_i are partition and protonation microconstants, respectively

$$p_i = \frac{c_i^o}{c_i} \qquad (10)$$

where I can be any of the indexes 00, 01, 10, and 11,

$$k_{01} = \frac{c_{01}}{[H^+]c_{00}} \qquad (11)$$

$$k_{10} = \frac{c_{10}}{[H^+]c_{00}} \qquad (12)$$

$$k_{11} = \frac{c_{11}}{[H^+]c_{01}} \qquad (13)$$

where c_i and c_i^o are the concentrations of microspecies i in water and octanol, respectively. The indexing method applied for microspecies and microconstants in Figure 2 and eqs 5–13 is described in the Appendix.

To create a model for predicting the distribution coefficient from experimental data, the determination of protonation and partition microconstants is not necessary. Moreover, the experimental determination of a part of the partition microconstants is very difficult or impossible when there are several protonation sites. However, apart from simpler cases, the structure-based prediction of the distribution coefficient has to be started at the level of the microspecies because generally macroconstants, like P_1 in eq 8 cannot be predicted directly.

Results and Discussion

Prediction of Protonation Microconstants—PrologD uses log k_i values predicted by pKalc,[25,26] another module of the Pallas system. The method, which is based on Hammett and Taft equations, is described by Perrin et al.[27] These equations have the following form:

$$\log k = \log k^0 - \rho \sum_{i=1}^{n} \sigma_i \qquad (14)$$

where k is the given microconstant, k^0 is the ionization constant for the parent compound (or protonation reaction center), ρ is a constant for the particular equilibrium (characteristic of the center), and σ is a constant characteristic of a given substituent (neutral or ionized) on a given position for the reaction. Since σ values can refer to charged substituents, the method is suitable to predict protonation microconstants (the original method applies corrections to calculate macroconstants from microconstants). The majority of log k, ρ, and σ values used by pKalc are taken from Perrin et al.,[27] while the rest have been calculated at CompuDrug.

The Prediction of the Partition Coefficient of the Uncharged Microspecies—PrologD uses the PrologP[28] module to predict the partition coefficient of the uncharged form of the compound (p_u). PrologP uses fragmentation methods that are based on the works of Rekker et al.,[29] Ghose et al.,[11,30], and Broto et al.,[12] which calculate log p_u as

$$\log p_u = \sum_{i=1}^{n} a_i f_i + \sum_{j=1}^{m} b_j F_j \qquad (15)$$

where p_u is the partition coefficient of the uncharged microspecies, f_i and F_j are the log P contributions of fragment (or atom) i and intramolecular interaction j, respectively, a_i and b_j are the incidences of fragment i and interaction j, respectively, and n and m are the number of the given type of fragments and interactions, respectively. Corrections applied in these methods for the prediction of the partition coefficient of zwitterions like α-amino acids were removed from the program because they were intended only for the calculation of macroconstants.

Prediction of the Partition Coefficients of Monovalent Ions—In the case of monoprotic substances the micro- and macrospecies are the same, which simplifies the prediction process. It is observed that both free ions and ion pairs can contribute to the partitioning of ionized species of

Table 1—Estimated Parameters for the Calculation Partition Coefficients of Ions

Type	eq[a]	a	b	c	d	n	s	F	R^2	R^2_{cv}[b]
Phenolates	22	0.807 (±0.041)	−0.234 (±0.033)	−0.331 (±0.192)	4.10 (±0.13)	49	0.198	312	0.954	0.946
Other anions	23	0.907 (±0.062)		−2.73 (±0.22)	4.58 (±0.28)	35	0.172	210	0.929	0.917
Cations	24	0.777 (±0.030)		−1.46 (±0.07)		29	0.197	745	0.965	0.957
Zwitterions	29	0.636 (±0.037)	−2.36 (±0.067)			37	0.258	281	0.889	0.875

[a] Equation used to calculate the parameters by regression analysis. [b] Calculated by the leave-one-out cross-validation method.[34]

Table 2—Compounds Used To Develop the log p Estimation Formula for Phenolate Ions

Name of Compound	Ref[a]	log p_u[b]	log k[b]	c_p/M[c]	log p_e[d]	log p_p[e]	Error[f]
2-Nitrophenol	32	1.89	7.23	0.05	−1.77	−1.65	−0.12
3-Methyl-2-nitrophenol	32	2.29	7.00	0.05	−1.60	−1.27	−0.33
4-Methyl-2-nitrophenol	32	2.37	7.63	0.05	−1.25	−1.30	0.05
5-Methyl-2-nitrophenol	32	2.31	7.34	0.05	−1.38	−1.31	−0.07
4-sec-Butyl-2-nitrophenol	32	3.84	7.59	0.05	0.10	0.02	0.08
4-Phenyl-2-nitrophenol	32	3.71	6.69	0.05	0.17	0.02	0.15
4-Methoxy-2-nitrophenol	32	2.02	7.40	0.05	−1.70	−1.57	−0.13
4-Chloro-2-nitrophenol	32	2.46	6.44	0.05	−0.89	−1.03	0.14
4-Chloro-5-methyl-2-nitrophenol	32	2.93	6.84	0.05	−0.37	−0.69	0.32
5-Fluoro-2-nitrophenol	32	1.91	6.30	0.05	−1.37	−1.47	0.10
4-(Trifluoromethyl)-2-nitrophenol	32	2.34	5.66	0.05	−0.60	−0.99	0.39
4-Formyl-2-nitrophenol	32	1.48	4.61	0.05	−1.76	−1.48	−0.28
2,4-Dinitrophenol	32	1.67	3.94	0.05	−1.18	−1.18	0.00
2,5-Dinitrophenol	32	1.80	5.18	0.05	−1.23	−1.34	0.11
6-methyl-2,4-dinitrophenol	32	2.12	4.31	0.05	−0.81	−0.89	0.08
4-nitrophenol	32	2.04	7.08	0.05	−1.76	−1.50	−0.26
3-Methyl-4-nitrophenol	32	2.48	7.33	0.05	−1.33	−1.16	−0.17
2-nitrophenol	32	1.89	7.23	0.01	−2.24	−2.03	−0.21
				0.02	−2.07	−1.90	−0.17
				0.06	−1.78	−1.60	−0.18
				0.11	−1.65	−1.38	−0.27
				0.21	−1.47	−1.13	−0.34
4-sec-Butyl-2-nitrophenol	32	3.84	7.59	0.01	−0.31	−0.18	−0.13
				0.02	−0.20	−0.12	−0.08
				0.06	0.12	0.06	0.06
				0.11	0.30	0.21	0.09
				0.21	0.50	0.42	0.08
4-Chloro-2-nitrophenol	32	2.46	6.44	0.01	−1.30	−1.43	0.13
				0.02	−1.11	−1.29	0.18
				0.06	−0.89	−0.97	0.08
				0.11	−0.60	−0.75	0.15
				0.21	−0.37	−0.50	0.13
4-(Trifluoromethyl)-2-nitrophenol	32	2.34	5.66	0.01	−1.22	−1.46	0.24
				0.02	−0.91	−1.29	0.38
				0.06	−0.63	−0.92	0.29
				0.11	−0.38	−0.69	0.31
				0.21	−0.14	−0.42	0.28
2,4-Dinitrophenol	32	1.67	3.94	0.01	−1.84	−1.79	−0.05
				0.02	−1.55	−1.54	−0.01
				0.06	−1.21	−1.10	−0.11
				0.11	−0.88	−0.85	−0.03
				0.21	−0.80	−0.58	−0.22
Pentachlorophenol	31	5.09	4.83	0.06	1.35	1.56	−0.21
				0.11	1.56	1.77	−0.21
				0.21	1.80	2.01	−0.21
2-Methyl-4,6-dinitrophenol	31	2.14	4.46	0.06	−0.70	−0.83	0.13
				0.11	−0.57	−0.59	0.02
				0.16	−0.50	−0.43	−0.07
				0.21	−0.41	−0.31	−0.10

[a] References to the publications where the experimental values can be found. [b] Experimental values. [c] The concentration of cations (Na$^+$ or K$^+$). [d] Experimental log p values. [e] Predicted log p values using eq 22 and experimental log p_u and log k values. [f] log p_e − log p_p.

compounds.[31,32] Assuming that ion pairing is negligible in the aqueous phase, the partition coefficient of a monovalent ion in the presence of a pairing ion can be expressed as

$$p = \frac{c^o_{ion} + c^o_{ip}}{c_{ion}} \qquad (16)$$

where c_{ion} is the concentration of the ionic species in the aqueous phase, c^o_{ion} and c^o_{ip} are the concentrations of the non-ion-paired and the ion-paired species in the octanol phase, respectively. Equation 16 can be rewritten as

$$p = p_{ion} + Ec_{pl} \qquad (17)$$

where c_{pl} is the concentration of the pairing ion in the aqueous phase. p_{ion} is defined as

$$p_{ion} = \frac{c^p_{ion}}{c_{ion}} \tag{18}$$

and E is the extraction constant[33]

$$E = \frac{c^p_{ip}}{c_{ion}c_{pl}} \tag{19}$$

Schwarzenbach et al.[32] recognized that, since the delocalization of the charge increases the lipophilicity of ions, the partitioning of dissociated phenols is affected by the strength of the electron withdrawing or donating effects of the substituents on the aromatic ring. It may be reasonable to use the protonation constant as a descriptor in the prediction of the partition coefficient of the ionized species because, in many cases, it correlates well with the charge delocalization effect for a given class of aromatic compounds. Based on the above assumption, eq 20 was established for the prediction of the extraction constant for dissociated phenols using

$$\log E = a \log p_u + b \log k + c \tag{20}$$

where p_u is the partition coefficient of the uncharged species, k is the protonation constant (log k = pK_a for monoprotic compounds), and a, b, and c are considered constants for a group of compounds. In the case of acids, log p_u − log p_{ion} was found to have small variance and therefore it is considered constant in our empirical model

$$\log p_{ion} = \log p_u - d \tag{21}$$

where d is a constant. Substituting eqs 20 and 21 into the logarithmic form of eq 17, eq 22 is obtained:

$$\log p = \log(10^{a\log p_u + b\log k + c}c_{pl} + 10^{\log p_u - d}) \tag{22}$$

For acids other than phenols, the equation was simplified to eq 23 by leaving out log k from the regression:

$$\log p = \log(10^{a\log p_u + c}c_{pl} + 10^{\log p_u - d}) \tag{23}$$

In the case of bases, assuming that $p_{ion} = 0$, the model was further simplified to eq 24:

$$\log p = a \log p_u + c + \log c_{pl} \tag{24}$$

The removal of log k from the regression equation established for these two groups of compounds was necessary because the available data were insufficient to subdivide the training sets based on the type of the ionizable center in the molecules (log p and log k are supposed to have different correlations in the various subclasses of acids and bases). In the case of bases, the reason for further simplification was that the collected data did not support the use of p_{ion} in the regression, probably because true p_{ion} values are too low compared with the p values. It should be noted that, for acids, using eq 24 has led to a considerable bias at low counterion concentrations.

Table 1 contains the parameters calculated by regression analysis and the results of cross-validation.[34] The experimental log p, log p_u and log k data, used to predict the parameters of eqs 22−24, were collected from the literature.[24,31−33,35−38] The training set and the prediction results are presented in Tables 2−4. The experiments were performed in the presence of Na$^+$, K$^+$, or Cl$^-$ as pairing ions

Table 3—Compounds Used To Develop the log p Estimation Formula for Monovalent Negative Ions Other Than Phenolates

Name of Compound	ref.[a]	log p_u[b]	c_{pl}/M[c]	log p_a[d]	log p_b[e]	error[f]
(2,4,5-Trichlorophenoxy)- acetic acid	31	3.31	0.06	−0.49	−0.78	0.29
			0.11	−0.32	−0.59	0.27
			0.16	−0.19	−0.45	0.26
			0.21	−0.11	−0.35	0.24
4-Chloro-α-(4-chlorophenyl)- benzeneacetic acid	31	4.64	0.06	0.55	0.47	0.08
			0.11	0.73	0.65	0.08
			0.16	0.80	0.78	0.02
			0.21	0.91	0.87	0.04
(2,4-Dichlorophenoxy)acetic acid	31	2.83	0.06	−0.86	−1.23	0.37
			0.11	−0.71	−1.03	0.32
			0.16	−0.60	−0.89	0.29
4-(2,4-Dichlorophenoxy)- butanoic acid	31	3.53	0.06	−0.41	−0.57	0.16
			0.11	−0.23	−0.38	0.15
			0.16	−0.09	−0.25	0.16
			0.21	−0.01	−0.15	0.14
3,6-Dichloro-2-methoxy- benzoic acid	31	2.49	0.06	−1.81	−1.55	−0.26
			0.11	−1.66	−1.34	−0.32
			0.16	−1.55	−1.21	−0.34
			0.21	−1.47	−1.10	−0.37
2,3,6-Trichlorobenzeneacetic acid	31	3.20	0.06	−1.17	−0.88	−0.29
			0.16	−0.85	−0.55	−0.30
			0.21	−0.79	−0.45	−0.34
2-(2,4,5-Trichlorophenoxy)- propanoic acid	31	3.80	0.06	−0.31	−0.32	0.01
			0.11	−0.13	−0.13	0.00
			0.16	0.01	0.00	0.01
			0.21	0.09	0.10	−0.01
Ketoprofen	36	3.12	0.01	−1.34	−1.32	−0.02
			0.05	−1.00	−1.01	0.01
			0.10	−0.83	−0.79	−0.04
			0.20	−0.47	−0.54	0.07
			0.25	−0.42	−0.46	0.04
Mefenamic acid	36	5.12	0.05	0.62	0.88	−0.26
			0.10	0.79	1.07	−0.28
			0.20	1.25	1.30	−0.05
			0.25	1.24	1.38	−0.14

[a] References to the publications where the experimental values can be found. [b] Experimental log p_u values (taken from ref 38 if not given in the reference cited). [c] The concentration of counterion (Na$^+$ or K$^+$). [d] Experimental log p values. [e] Predicted log p values using eq 23 and experimental log p_u values. [f] log p_a − log p_b.

at such pH values that the uncharged species had no considerable effect on log D. This condition is satisfied if pH ≥ pK_a + 4 in the case of acids, and if pH ≤ pK_a − 4, in the case of bases. Counterions other than Na$^+$, K$^+$, and Cl$^-$ were not present in significant quantities, so they had no effect on the partitioning of the examined ions. Since experimental values used were determined at 25 °C (except when noted) and at ionic strengths ranging from 0.01 to 0.25M, the prediction is most effective under such conditions. Equations 22−24 are not valid at c_{pl} = 0 because the concentrations of negative and positive ions have to be equal in both phases.

It is probable that log k could be included also with other aromatic acids and bases like benzoic acids, anilines, and pyridines, but additional data need to be collected for these types of compounds before setting up such regression equations.

Prediction of the Partition Coefficient of the Zwitterionic Microspecies—Rewriting eq 8 for amphoteric compounds, we get

$$P = \frac{pk_{01} + p_u k_{10}}{k_{01} + k_{10}} \tag{25}$$

where P is the partition coefficient of the neutral macrospecies and p and p_u are the partition coefficients of the zwitterionic and uncharged microspecies, respectively. As can be seen

Table 4—Compounds Used To Develop log p Estimation Formula for Monovalent Positive Ions

Name of Compound	Ref.[a]	log p_u[b]	c_p/M[c]	log p_e[d]	log p_p[e]	error[f]
Promazine	33	4.55	0.125	1.04[h]	1.17	−0.13
Chlorpromazine	33	5.40[g]	0.125	1.51[h]	1.83	−0.32
Triflupromazine	33	5.19	0.125	1.78[h]	1.67	0.11
Phenylmethylamine	37	1.03	0.1	−2.13	−1.66	−0.47
Phenylethylamine	37	1.37	0.1	−1.59	−1.39	−0.20
Phenylpropylamine	37	1.83	0.1	−1.13	−1.04	−0.09
Phenylbutylamine	37	2.39	0.1	−0.70	−0.60	−0.10
Phenylpentylamine	37	2.90	0.1	−0.18	−0.21	0.03
4-Pyridylpropylamine	37	0.49	0.1	−2.03[i]	−2.08	0.05
4-Pyridylbutylamine	37	0.86	0.1	−1.76[i]	−1.79	0.03
4-Pyridylpentylamine	37	1.32	0.1	−1.39[i]	−1.43	0.04
Trimethoprim	36	0.91	0.05	−2.49	−2.05	−0.44
			0.075	−1.90	−1.88	−0.02
			0.1	−1.65	−1.75	0.10
			0.15	−1.39	−1.58	0.19
			0.2	−1.16	−1.45	0.29
Propranolol	24	3.09	0.15	0.50	0.12	0.38
4-Methylaniline	32, 35	1.40	0.09	−1.55	−1.42	−0.13
			0.14	−1.32	−1.23	−0.09
			0.19	−1.17	−1.09	−0.08
			0.24	−1.08	−0.99	−0.09
3,4-Dimethylaniline	32, 35	1.84	0.09	−1.02	−1.08	0.06
			0.14	−0.80	−0.88	0.09
			0.19	−0.66	−0.75	0.09
			0.24	−0.57	−0.65	0.08
2,4,5-Trimethylaniline	32, 35	2.27	0.09	−0.61	−0.74	0.14
			0.14	−0.38	−0.55	0.17
			0.19	−0.25	−0.42	0.17
			0.24	−0.15	−0.32	0.16

[a] References to the publications where the experimental values can be found. [b] Experimental log p_u values (taken from ref 38 if not given in the reference cited). [c] The concentration of counterion (Cl⁻). [d] Experimental log p values. [e] Predicted log p values using eq 24 and experimental log p_u values. [f] log p_e − log p_p. [g] Predicted from experimental data measured at 30 °C, pH 6.6. [h] Measured at 30 °C. [i] Predicted from experimental data measured at pH 7.

from eq 25, P is the weighted average of two partition microconstants. Provided that

$$\log p_u + \log p \approx 2 \qquad (26)$$

and

$$\log k_{01} - \log k_{10} < 0.7 \qquad (27)$$

the error caused by ignoring the partitioning of the zwitterionic species is less than 5% in the calculation of p_u from P. Takács-Novák et al.[21−23] neglected the partitioning of the zwitterionic microspecies when they calculated the partition coefficient of the uncharged microspecies of some zwitterionic compounds from experimentally determined protonation microconstants and log D data. On the other hand, the effect of the uncharged microspecies on P is not significant if

$$\log k_{01} - \log k_{10} > 3.3 \qquad (28)$$

If the condition given in eq 28 is met, then the partition coefficient of the zwitterionic microspecies is approximately equal to the partition coefficient of neutral macrospecies, which corresponds to the distribution coefficient measured at the isoelectric point.

Assuming that there is a correlation between the logarithm of partition coefficient of the uncharged species and that of the zwitterionic species, we used the following formula for the prediction

$$\log p = a \log p_{u,pred} + b \qquad (29)$$

Table 5—Compounds Used To Develop the log p Estimation Formula for Zwitterions

Name of Compound	Ref[a]	log p_u[b]	log p_e[c]	log p_p[d]	error[e]
Sarcosine	39	−0.69	−2.78	−2.80	0.02
Ala	39	−0.48	−2.77	−2.67	−0.10
Cys	39	−0.08	−2.55	−2.41	−0.14
Gly	39	−1.00	−3.00	−3.00	0.00
His	39	−0.89	−2.85	−2.93	0.08
Ile	39	1.08	−1.80	−1.68	−0.12
Leu	39	1.08	−1.72	−1.68	−0.04
Met	39	0.05	−2.10	−2.33	0.23
Phe	39	1.18	−1.44	−1.61	0.17
Pro	39	−0.02	−2.62	−2.37	−0.25
Ser	39	−1.57	−3.00	−3.36	0.36
Thr	39	−1.05	−2.83	−3.03	0.20
Trp	39	1.35	−1.15	−1.50	0.35
Tyr	39	0.66	−2.11	−1.94	−0.17
Val	39	0.56	−2.29	−2.01	−0.28
Phe-Leu	40	2.19	−1.07	−0.97	−0.10
Leu-Phe	40	2.19	−1.12	−0.97	−0.15
Phe-Phe	40	2.29	−0.82	−0.91	0.09
Leu-Leu	40	2.09	−1.46	−1.03	−0.43
Trp-Trp	40	2.62	−0.12	−0.70	0.58
Trp-Phe	40	2.46	−0.40	−0.80	0.40
Trp-Ala	40	0.80	−1.85	−1.85	0.00
Trp-Leu	40	2.36	−0.63	−0.86	0.23
Phe-Pro	40	1.06	−1.23	−1.69	0.46
Pro-Phe	40	1.10	−2.07	−1.66	−0.41
Phe-Phe-Phe	40	3.41	0.13	−0.20	0.33
Gly-Phe-Phe	40	1.23	−1.21	−1.58	0.37
Phe-Val-Gly	40	0.61	−2.33	−1.97	−0.36
Phe-Val-Phe	40	2.79	−0.62	−0.59	−0.03
Phe-Val-Ala	40	1.13	−2.01	−1.64	−0.37
Leu-Leu-Leu	40	3.10	−0.92	−0.39	−0.53
Trp-Gly-Gly	40	−0.78	−2.65	−2.86	0.21
Trp-Phe-Ala	40	1.91	−0.90	−1.15	0.25
Trp-Trp-Leu	40	3.96	0.60	0.15	0.45
Pro-Leu-Leu	40	2.01	−1.62	−1.08	−0.54
Leu-Pro-Leu	40	1.97	−1.52	−1.11	−0.41
Leu-Leu-Pro	40	1.97	−1.47	−1.11	−0.36

[a] References to the publications where the experimental values and the structures of compounds can be found. [b] Predicted log p_u values using Rekker's method.[29] [c] Experimental log p values. [d] Predicted log p values using eq 29. [e] log p_e − log p_p.

where $p_{u,pred}$ is the predicted partition coefficient of the uncharged microspecies and a and b are parameters calculated by regression analysis. Experimental log D data of α-amino acids and peptides[38−40] listed in Table 5 were used to calculate parameters a and b in eq 29. Substituting the predicted log k values of these compounds into eq 28, it can be shown that the partitioning of the uncharged species can be neglected around the isoelectric point. Rekker's method[29] was applied for calculating log $p_{u,pred}$. Table 1 contains the results of regression.

Compounds with Several Ionizable Groups—In more complex cases, when several ionizable groups coexist in a molecule, log p of the microspecies with several charges is calculated step by step. In each step the log p value of a microspecies is predicted by eqs 22, 23, 24, or 29, but instead of log p_u, the log p value of another microspecies is applied in which the charge is lower by one.

Example: Prediction of log D for Leu-Phe—To illustrate the suggested technique, Leu-Phe was selected as an example compound for the prediction of log D. pH−partition profiles predicted at different counterion concentrations and experimental log D values[40] are shown in Figure 3. The curves were calculated by eqs 2 and 5−9 using predicted microconstants that are shown in Table 6 (see Figures 1 and 2 for the indexing of the constants).
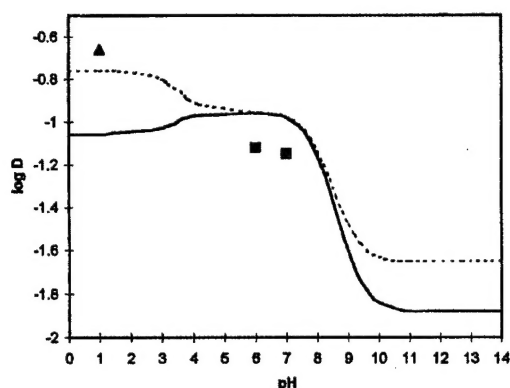
Figure 3—Predicted pH—partition profiles and experimental log D values of Leu-Phe: predicted values at $[Na^+] = [Cl^-] = 0.05$ M (continous line) and at $[Na^+] = [Cl^-] = 0.1$ M (dashed line), observed values in 0.1 M HCl (triangle) and in 0.1 M phosphate buffer (square).

Table 6—Predicted Constants Used for the log D Prediction of Leu-Phe

Constant	Value	Eq[a]	Comment
log k_{01}	8.14	14	Taft equation[27]
log k_{10}	3.44	14	Taft equation[27]
log k_{11}	3.44	14	Taft equation[27]
log p_{00}	−1.65	23	at $[Na^+] = 0.1$ M
log p_{00}	−1.88	23	at $[Na^+] = 0.05$ M
log p_{01}	−0.97	29	
log p_{10}	2.19	15	Rekker method[29] (log $p_2 = $ log p_u)
log p_{11}	−0.76	24	at $[Cl^-] = 0.1$ M
log p_{11}	−1.06	24	at $[Cl^-] = 0.05$ M

[a] Equation used for the prediction of the microconstant.

Conclusion

It is possible to predict the log D value of a compound containing several ionizable groups at a given pH and ion-pair (Na^+, K^+, or Cl^-) concentration from the topological structure of the molecule by a general method.

Steps of the prediction are as follows:

1. The micro protonation constants are predicted using eq 14.

2. The micropartition coefficients are predicted by eqs 15, 22−24, and 29.

3. The macroprotonation constants and the macropartition coefficients are calculated by eqs 34 and 35, respectively.

4. log D is calculated by eq 37.

The algorithm was evaluated using a test set of 220 log D data of 84 compounds. The results of testing are reported in ref 41.

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